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```
=> s (serum albumin)
    574652 SERUM
    101650 ALBUMIN
L1    68146 (SERUM ALBUMIN)
      (SERUM(W)ALBUMIN)

=> s l1 and (CYS-34 or cysteine 34)
    12759 CYS
    185429 34
      83 CYS-34
      (CYS(W)34)
    63438 CYSTEINE
    185429 34
      30 CYSTEINE 34
      (CYSTEINE(W)34)
L2    74 L1 AND (CYS-34 OR CYSTEINE 34)

=> s l2 and py<2000
    12397709 PY<2000
L3    39 L2 AND PY<2000

=> d l3,cbib,ab,1-39
```

L3 ANSWER 1 OF 39 MEDLINE on STN
2000210399. PubMed ID: 10746304. Comparison of formats for the development of fiber-optic biosensors utilizing sol-gel derived materials entrapping fluorescently-labelled protein. Flora K; Brennan J D. (Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.) Analyst, (1999 Oct) 124 (10) 1455-62. Journal code: 0372652. ISSN: 0003-2654. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of fiber-optic biosensors requires that a biorecognition element and a fluorescent reporter group be immobilized at or near the surface of an optical element such as a planar waveguide or optical fiber. In this study, we examined a model biorecognition element-reporter group couple consisting of human **serum albumin** that was site-selectively labelled at **Cys 34** with iodoacetoxymethyl-nitrobenzoxymethyl (HSA-NBD). The labelled protein was encapsulated into sol-gel derived materials that were prepared either as monoliths, as beads that were formed at the distal tip of a fused silica optical fiber, or as thin films that were dipcast along the length of a glass slide or optical fiber. For fiber-based studies, the entrapped protein was excited using a helium-cadmium laser that was launched into a single optical fiber, and emission was separated from the incident radiation using a perforated mirror beam-splitter, and detected using a monochromator-photomultiplier tube assembly. Changes in fluorescence intensity were generated by denaturant-induced conformational changes in the protein or by iodide quenching. The analytical parameters of merit for the different encapsulation formats, including minimum protein loading level, response time and limit-of-detection, were examined, as were factors such as protein accessibility, leaching and photobleaching. Overall, the results indicated that both beads and films were suitable for biosensor development. In both formats, a substantial fraction of the entrapped protein remained accessible, and the entrapped protein retained a large degree of conformational flexibility. Thin films showed the most rapid response times, and provided good detection limits for a model analyte. However, the entrapment of proteins into beads at the distal tip of fibers provided better signal-to-noise and signal-to-background ratios, and required less protein for preparation. Hence, beads appear to be the most viable method for interfacing of proteins to optical fibers.

L3 ANSWER 2 OF 39 MEDLINE on STN
2000034160. PubMed ID: 10568165. Interaction of acrylodan with human **serum albumin**. A fluorescence spectroscopic study. Moreno F; Cortijo M; Gonzalez-Jimenez J. (Departamento de Quimica-Fisica Farmaceutica, Facultad de Farmacia U.C.M., Madrid, Spain.) Photochemistry and photobiology, (1999 Nov) 70 (5) 695-700. Journal code: 0376425. ISSN: 0031-8655. Pub. country: United States. Language: English.

AB The binding of the fluorescent probe acrylodan (AC) to human **serum albumin** (HSA) was studied by fluorescence spectroscopy. The binding

isotherms could be fitted to two types of sites. Competition experiments using iodoacetamide suggested that AC binds tightly on HSA by the **cysteine-34**. Attempts were made to find the location of the second site using high concentrations of warfarin, phenylbutazone, diazepam, indomethacin, palmitic acid or bilirubin in order to displace the bound AC to the HSA. Bilirubin was the only ligand able to displace the bound AC. This result suggests that AC, which is a very hydrophobic molecule also capable of labeling lysine residues, should also bind the human albumin in the primary site of bilirubin, but with less affinity than to the **cysteine-34**.

L3 ANSWER 3 OF 39 MEDLINE on STN

1999331485. PubMed ID: 10403053. Irreversible adsorption of human **serum albumin** to hydrogel contact lenses: a study using electron spin resonance spectroscopy. Garrett Q; Griesser H J; Milthorpe B K; Garrett R W. (Cooperative Research Centre for Eye Research and Technology, University of New South Wales, Sydney, Australia.. q.garrett@cclru.unsw.edu.au) . Biomaterials, (1999 Jul) 20 (14) 1345-56. Journal code: 8100316. ISSN: 0142-9612. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Human **serum albumin** (HSA) was specifically spin labelled with 4-maleimido-tempo (MSL) at its **cysteine 34** residue (HSA-MSL). The irreversible adsorption of HSA-MSL to hydrogel contact lenses (etafilcon A, tefilcon and vifilcon A) was investigated using electron spin resonance (ESR) spectroscopy. Changes in ESR spectral characteristics of adsorbed HSA-MSL as compared to HSA-MSL in solution displayed an additional immobilisation of the spin label due to the adsorption. This immobilisation of MSL corresponds to a large conformational alteration of the HSA-MSL near the modified **Cys 34** residue. For both etafilcon A and tefilcon, the rate of irreversible adsorption was relatively slow compared with that of vifilcon A where the maximum state of immobilisation and hence conformational change occurred within the first hour of adsorption. Furthermore, tefilcon produced markedly different ESR spectra where a strong conformational change to a less mobile protein was apparent. This supported a model where the direct irreversible adsorption of HSA from solution dominated on tefilcon as opposed to conversion of the adsorbed protein from the reversible to the irreversible state on both etafilcon A and vifilcon A. HSA-MSL adsorption onto hydrophobic poly(methylmethacrylate) (PMMA) and hydrophilic poly(N-ter-butylacrylamide) (PTBAM) latex beads was also investigated. The spin label MSL was found to be less mobile when HSA was adsorbed onto PMMA compared with PTBAM beads. It was also found that the rate of irreversible adsorption of HSA is far higher onto PMMA surfaces than onto PTBAM surfaces.

L3 ANSWER 4 OF 39 MEDLINE on STN

1999238388. PubMed ID: 10219106. Investigation of slow dynamics of the sulfhydryl in the solution and gel states of bovine **serum albumin**: A vector electron paramagnetic resonance study. Hayashi T; Shimoyama Y; Kuwata K; Era S. (Department of Physiology, Gifu University School of Medicine, Gifu, 500-8705, Japan.) Japanese journal of physiology, (1999 Feb) 49 (1) 27-33. Journal code: 2985184R. ISSN: 0021-521X. Pub. country: Japan. Language: English.

AB **Serum albumin** has one reactive sulfhydryl (**Cys-34**) that is one of the important binding sites. **Cys-34** is located in the crevice on the surface of the albumin molecule and is therefore restricted in its motion. Bovine **serum albumin** (BSA) Fraction V forms a transparent gel at pD 4.0 (F-form) in D2O at protein concentrations above 7% (BSA*-gel). We studied the molecular motion of **Cys-34** on BSA in the solution and gel states by the vector electron paramagnetic resonance (EPR) method using a maleimide spin label. The rotational correlation times of the spin label bound to **Cys-34** in the BSA solution and BSA*-gel were in the order of $10(-6)$ and $10(-5)$ s, respectively. A longer rotational correlation time of the **Cys-34** spin label in the BSA*-gel suggested that the gel network formed in BSA may drastically slow the motion of **Cys-34**. The integrated value obtained from the vector EPR spectra also showed an extremely dramatic slowing of the **Cys-34** spin label during the gel formation. On the other hand, the values for order parameter and the inclination of the principal axis (z) of the **Cys-34** spin label to the rotational axis (μ) were the same in the BSA solution and BSA*-gel.

L3 ANSWER 5 OF 39 MEDLINE on STN

1999091622. PubMed ID: 9873023. Kinetics of peroxynitrite reaction with amino acids and human **serum albumin**. Alvarez B; Ferrer-Sueta G; Freeman B A; Radi R. (Laboratorio de Enzimologia, Unidad Asociada Enzimologia, Universidad de la Republica, 11800 Montevideo, Uruguay.) Journal of biological chemistry, (1999 Jan 8) 274 (2) 842-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB An initial rate approach was used to study the reaction of peroxynitrite with human **serum albumin** (HSA) through stopped-flow spectrophotometry. At pH 7.4 and 37 degreesC, the second order rate constant for peroxynitrite reaction with HSA was $9.7 \pm 1.1 \times 10(3) \text{ M}^{-1} \text{ s}^{-1}$. The rate constants for sulfhydryl-blocked HSA and for the single sulfhydryl were 5.9 ± 0.3 and $3.8 \pm 0.8 \times 10(3) \text{ M}^{-1} \text{ s}^{-1}$, respectively. The corresponding values for bovine **serum albumin** were also determined. The reactivity of sulfhydryl-blocked HSA increased at acidic pH, whereas plots of the rate constant with the sulfhydryl versus pH were bell-shaped. The kinetics of peroxynitrite reaction with all free L-amino acids were determined under pseudo-first order conditions. The most reactive amino acids were cysteine, methionine, and tryptophan. Histidine, leucine, and phenylalanine (and by extension tyrosine) did not affect peroxynitrite decay rate, whereas for the remaining amino acids plots of kobs versus concentration were hyperbolic. The sum of the contributions of the constituent amino acids of the protein to HSA reactivity was comparable to the experimentally determined rate constant, where cysteine and methionine (seven residues in 585) accounted for an estimated 65% of the reactivity. Nitration of aromatic amino acids occurred in HSA following peroxynitrite reaction, with nitration of sulfhydryl-blocked HSA 2-fold higher than native HSA. Carbon dioxide accelerated peroxynitrite decomposition, enhanced aromatic amino acid nitration, and partially inhibited sulfhydryl oxidation of HSA. Nitration in the presence of carbon dioxide increased when the sulfhydryl was blocked. Thus, **cysteine 34** was a preferential target of peroxynitrite both in the presence and in the absence of carbon dioxide.

L3 ANSWER 6 OF 39 MEDLINE on STN

1998278978. PubMed ID: 9614070. Cisplatin binding sites on human albumin. Ivanov A I; Christodoulou J; Parkinson J A; Barnham K J; Tucker A; Woodrow J; Sadler P J. (Department of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ, United Kingdom.) Journal of biological chemistry, (1998 Jun 12) 273 (24) 14721-30. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Reactions of cisplatin (cis-[PtCl₂(NH₃)₂]) with albumin are thought to play an important role in the metabolism of this anticancer drug. They are investigated here via (i) labeling of cisplatin with ¹⁵N and use of two-dimensional ¹H,¹⁵N NMR spectroscopy, (ii) comparison of natural human **serum albumin** with recombinant human albumin (higher homogeneity and SH content), (iii) chemical modification of Cys, Met, and His residues, (iv) reactions of bound platinum with thiourea, and (v) gel filtration chromatography. In contrast to previous reports, it is shown that the major sulfur-containing binding site involves Met and not **Cys-34**, and also a N ligand, in the form of an S,N macrochelate. Additional monofunctional adducts involving other Met residues and **Cys-34** are also observed. During the later stages of reactions of cisplatin with albumin, release of NH₃ occurs due to the strong trans influence of Met sulfur, which weakens the Pt-NH₃ bonds, and protein cross-linking is observed. The consequences of these findings for the biological activity of cisplatin-albumin complexes are discussed.

L3 ANSWER 7 OF 39 MEDLINE on STN

1998246218. PubMed ID: 9586806. Photodynamically generated bovine **serum albumin** radicals: evidence for damage transfer and oxidation at cysteine and tryptophan residues. Silvester J A; Timmins G S; Davies M J. (Department of Chemistry, University of York, UK.) Free radical biology & medicine, (1998 Mar 15) 24 (5) 754-66. Journal code: 8709159. ISSN: 0891-5849. Pub. country: United States. Language: English.

AB Porphyrin-sensitized photooxidation of bovine **serum albumin** (BSA) results in oxidation of the protein at (at least) two different, specific sites: the **Cys-34** residue giving rise to a thiyl radical (RS.); and one or both of the tryptophan residues (Trp-134 and Trp-214) resulting in the formation of tertiary carbon-centred radicals and disruption of the tryptophan ring system. In the case of porphyrins such as hematoporphyrin, which bind at specific sites on BSA, these species appear to arise via long-range transfer of damage within the protein structure, as the binding site is some distance from the ultimate site of radical formation. This transfer of damage is shown to depend on a number of factors including the conformation of the protein, the presence of blocking groups and pH. Alteration of the protein conformation results in radical formation at additional (or alternative) sites, as does blocking of the preferred loci of radical formation. The formation of these thiyl and tryptophan-derived radicals does not lead to significant aggregation or fragmentation of the protein, though it does result in a dramatic enhancement in the susceptibility of the oxidised protein to proteolytic degradation by a range of proteases. The generation of protein-derived radicals also results in an enhancement of photobleaching of the porphyrin, suggesting that protein radical generation is linked to porphyrin photooxidation.

L3 ANSWER 8 OF 39 MEDLINE on STN
1998141963. PubMed ID: 9473299. Protein hydroperoxides and carbonyl groups generated by porphyrin-induced photo-oxidation of bovine **serum albumin**. Silvester J A; Timmins G S; Davies M J. (Department of Chemistry, University of York, York, United Kingdom.) Archives of biochemistry and biophysics, (1998 Feb 15) 350 (2) 249-58. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB Porphyrin-sensitized photo-oxidation of bovine **serum albumin** results in oxidation at specific sites to produce protein radical species: at the **Cys-34** residue (to give a thiyl radical) and at one or both tryptophan residues (Trp-134 and Trp-214) to give tertiary carbon-centered radicals and cause disruption of the indole ring system. This study shows that these photo-oxidation processes also consume oxygen and give rise to hydrogen peroxide, protein hydroperoxides, and carbonyl functions. The yield of hydrogen peroxide, protein hydroperoxides, and carbonyl functions is shown to be dependent on illumination time, the nature of the sensitizer, and the concentration of oxygen; the yield of hydroperoxides can also be markedly diminished by the presence of a spin trap which reacts with the initial protein radicals. The mechanism of formation of the protein hydroperoxides is suggested to be primarily through type I processes (i.e., independent of singlet oxygen), while type II (singlet oxygen) mechanisms may play a significant role in protein carbonyl formation. Reaction of the protein hydroperoxide species with metal ion complexes is shown to produce further protein-derived radicals which are predominantly present on amino acid side chains.
Copyright 1998 Academic Press.

L3 ANSWER 9 OF 39 MEDLINE on STN
97480748. PubMed ID: 9339393. pH-dependent regulation of leukocyte 5-lipoxygenase activity in inflammatory exudates by albumin. Benz M; Werz O; Jacob R; Steinhilber D. (Institute of Pharmaceutical Chemistry, University of Frankfurt, Germany.) Inflammation research : official journal of the European Histamine Research Society ... [et al.], (1997 Sep) 46 (9) 366-72. Journal code: 9508160. ISSN: 1023-3830. Pub. country: Switzerland. Language: English.

AB OBJECTIVE AND DESIGN: In order to study the regulation of cellular 5-lipoxygenase activity under inflammatory conditions, the effects of inflammatory exudates on rat leukocyte 5 lipoxygenase activity were investigated. MATERIALS: Peritoneal leukocytes and inflammatory exudates were collected from glycogen treated rats. TREATMENT: Glycogen (1 g/kg body weight, in a final volume of 3 ml PBS) was injected intraperitoneally into male Wistar rats. After 4 h, the inflammatory exudate was collected. METHODS: Rat peritoneal leukocytes were isolated and the cellular 5-lipoxygenase activity was determined by HPLC after cell stimulation with calcium ionophore A23187. RESULTS: Inflammatory exudates from glycogen treated animals strongly inhibited cellular 5-lipoxygenase activity of ionophore challenged leukocytes. Albumin was identified as the inhibitor in exudates. Inhibition of cellular 5-lipoxygenase activity by albumin was pH-dependent and was strongly increased by the alkaline pH (7.9-8.0) of the exudate. The albumin effect increased in the range of pH 7.4-8.2 where albumin undergoes a conformational change called neutral to base (N-B) transition. S-Carboxymethyl-albumin had a similar activity to that of albumin, which indicated that the free SH-group at **Cys-34** of albumin is not necessary for the effect. The albumin dimer showed a significantly higher inhibition than albumin and it suppressed cellular 5-lipoxygenase activity by 98%. Peptic and tryptic fragments of albumin which comprise domains I, II and II, III, respectively, were less active or inactive. Thus, an intact albumin molecule or the dimer are required for efficient inhibition of cellular 5-lipoxygenase activity. CONCLUSIONS: Our data suggest that during inflammation, albumin extravasation and changes in pH-value are involved in the regulation of the inflammatory reaction by suppression of leukotriene release.

L3 ANSWER 10 OF 39 MEDLINE on STN
97452481. PubMed ID: 9308895. Effect of nitric oxide on the ligand-binding activity of albumin. Kashiba-Iwatsuki M; Miyamoto M; Inoue M. (Department of Biochemistry, Osaka City University Medical School, Osaka, Japan.) Archives of biochemistry and biophysics, (1997 Sep 15) 345 (2) 237-42. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB The redox state of the **Cys-34** on albumin plays an important role in ligand binding of this plasma protein. We previously reported that mixed-disulfide formation of albumin with low molecular weight thiols, such as cysteine and glutathione, increased the affinity of this protein for phenolsulfophthalein (PSP) and Cu(II). Although nitric oxide (NO) and its metabolites easily react with various thiols, including that of albumin, and form S-nitrosothiol derivatives, the effect of such

modification on the ligand-binding activity of this plasma protein remains to be elucidated. Kinetic analysis revealed that S-nitrosylation of **Cys-34** on bovine **serum albumin** (BSA) decreased its binding activity for PSP. NO also decreased the ligand-binding activity of fresh plasma samples from rat and human. S-nitrosylation also decreased the binding activity of BSA for Cu(II). These results indicate that reversible modification of the **Cys-34** by NO and oxidative stress might play regulatory roles in the binding and transport of organic anions and heavy metals in the circulation.

L3 ANSWER 11 OF 39 MEDLINE on STN

97356266. PubMed ID: 9212708. Characterization of the glycation of albumin in freeze-dried and frozen human serum. Bunk D M. (National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA.) Analytical chemistry, (1997 Jul 1) 69 (13) 2457-63. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB Human **serum albumin** (HSA) in fresh frozen and freeze-dried serum reference materials was examined by mass spectrometry and a variety of affinity chromatography techniques. The relative molecular mass distribution of HSA in fresh frozen serum was found to be identical to that of an HSA standard. However, the HSA in the freeze-dried reference serum exhibited a relative molecular mass distribution that was shifted to higher mass, broader, and substantially more heterogeneous than that of HSA in fresh frozen serum. A proteolytic cyanogen bromide digestion of the HSA from freeze-dried serum contained adducts approximately 162 u higher in mass than digest fragments 124-298 and 447-548, suggesting glycation. The presence of glycation on fragments 124-298 and 447-548 correlates with the known sites of HSA glycation. Glycation was further confirmed by the mass spectral analysis of the retained and unretained fractions from glycoaffinity chromatography of HSA from freeze-dried serum. The relative molecular weight of the HSA in the retained fraction indicated the presence of a doubly glycated species. The chemical heterogeneity of **Cys-34**, the site of the only free thiol in HSA, was examined and found not to be a substantial source of molecular mass heterogeneity for HSA from either fresh frozen or freeze-dried serum.

L3 ANSWER 12 OF 39 MEDLINE on STN

97321115. PubMed ID: 9177846. Structural determination of the conjugate of human **serum albumin** with a mitomycin C derivative, KW-2149, by matrix-assisted laser desorption/ionization mass spectrometry. Yasuzawa T; Tomer K B. (Laboratory of Structural Biology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.) Bioconjugate chemistry, (1997 May-Jun) 8 (3) 391-9. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB A new mitomycin C derivative, KW-2149, is known to form a covalent conjugate with human **serum albumin** (HSA). This conjugate exhibits 1/20 of the anticellular activity of unconjugated KW-2149. Structural studies of this conjugate were carried out using a combination of enzymatic digestion, high-performance liquid chromatography (HPLC), and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The tryptic peptide T5 (residues 21-41) was the only peptide found to be modified by KW-2149 moieties, the [(gamma-L-glutamylamino)ethyl]thio group or the (2-aminoethyl)thio group, through a disulfide bond. Although the latter peptide lost its mitomycin C moiety in the course of tryptic digestion, these data strongly suggest that KW-2149 was bound to **Cys-34**, the only free cysteine on HSA.

L3 ANSWER 13 OF 39 MEDLINE on STN

97273972. PubMed ID: 9128146. Probing the **cysteine 34** residue in human **serum albumin** using fluorescence techniques. Narazaki R; Maruyama T; Otagiri M. (Faculty of Pharmaceutical Sciences, Kumamoto University, Japan.) Biochimica et biophysica acta, (1997 Apr 4) 1338 (2) 275-81. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB The microenvironment surrounding **Cys-34** in human **serum albumin** (HSA) has been studied using acrylodan, a cysteine-specific fluorescence probe. Fluorescence emission maximum (Em(max)) of acrylodan covalently attached to **Cys-34** was observed at 476 nm, which was nearly the same as for acetonitrile. The Em(max) exhibited a shift toward longer wavelength with oleate binding. The acrylodan fluorescence of HSA exhibited heterogeneous decay kinetics, which adequately fit a double-exponential decay model. When three or more oleate molecules were bound to one albumin molecule, the fractional intensity was shifted in favor of the shorter lived component. These results suggest that oleate binding induces a conformational or dynamic change which is localized in the **Cys-34** region. Unfolding studies with guanidine-HCl indicate that **Cys-34** appears to be located on the surface of HSA molecule and that it is protected by adjacent amino acid residues. Solvent accessibility of

acrylodan with HSA in the absence and presence of oleate was determined from acrylamide quenching, and suggests that oleate binding enhances the solvent exposure of the acrylodan fluorophore. In order to determine the nature of the electrostatic potential near **Cys-34**, the quenching rate constants for anionic (iodide) and cationic (thallium) quenchers were determined as a function of ionic strength of solvent. The ionic strength dependence of quenching indicated that there was an electrostatic attractive force between the fluorophore and both ionic quenchers. These results are consistent with a model in which amphoteric charges which arise from charged amino acid residues were surrounding **Cys-34**. Interestingly, oleate binding resulted in changes in the spatial relationships between acrylodan and these charged residues. Thus, the experiments described herein provide the information concerning an oleate-induced alteration in the nature of the local environment surrounding **Cys-34** and suggests that long chain fatty acid binding provides a method for regulating the radical-trapping antioxidant activity of **Cys-34** in HSA in vivo.

L3 ANSWER 14 OF 39 MEDLINE on STN

96390393. PubMed ID: 8797379. Accessibility of the fluorescent reporter group in native, silica-adsorbed, and covalently attached acrylodan-labeled serum albumins. Ingersoll C M; Jordan J D; Bright F V. (Department of Chemistry, State University of New York at Buffalo 14260-3000, USA.) Analytical chemistry, (1996 Sep 15) 68 (18) 3194-8. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB Fluorescence quenching techniques are used to investigate the accessibility of a model biorecognition element-reporter group system when in buffer, surface-adsorbed, and covalently attached to a silica surface. The site-selective fluorescent reporter group, 6-acryloyl(dimethylamino)naphthalene (acrylodan, Ac), is attached covalently (at **cysteine-34**) to bovine and human **serum albumin** (BSA and HSA, respectively) and serves as a surrogate recognition element-reporter group system. Molecular oxygen is used to quench the Ac fluorescence and the accessibility, in the form of bimolecular rate constants (kq), in each model system is quantified. Although one might expect these systems to exhibit similar behavior, differences in quenching characteristics are observed, such as wavelength dependency of the Stern-Volmer quenching constant (KSV) for the native proteins in buffer. BSA-Ac exhibits wavelength dependent KSV values as well as a blue-shifted emission spectrum on O2 addition. Physisorption of BSA-Ac onto a fused-silica optical fiber lowers the accessibility of Ac to O2, whereas covalent attachment of BSA-Ac to APTES/glutaraldehyde-modified silica enhances the accessibility of the Ac reporter group to O2.

L3 ANSWER 15 OF 39 MEDLINE on STN

96299075. PubMed ID: 8660549. S-nitrosation of **serum albumin**: spectrophotometric determination of its nitrosation by simple S-nitrosothiols. Zhang H; Means G E. (Department of Biochemistry, Ohio State University, 484 West 12th Avenue, Columbus, Ohio, 43210, USA.) Analytical biochemistry, (1996 May 15) 237 (1) 141-4. Journal code: 0370535. ISSN: 0003-2697. Pub. country: United States. Language: English.

AB The transfer of nitroso groups from S-nitroso-L-cysteine (1) and six other simple S-nitrosothiols to **Cys 34** of bovine **serum albumin** (2) has been followed using Ellman's reagent, 5,5'-dithio-bis (2-nitrobenzoate) (3), to detect the resulting thiols. The described method utilizes the low reactivity of (3) with (2) and the high extinction coefficient of 2-nitro-5-thiobenzoate produced upon its reaction with thiols to follow the transfer of nitroso moieties at low concentrations where other procedures are not feasible. A second-order rate constant of 6400 M⁻¹ s⁻¹ obtained for the reaction of (2) with S-nitrosomercaptoethylamine is approximately 10 times faster than that for its reaction with (1), approximately 40 times faster than that for its reaction with S-nitroglutathione, and consistent with **Cys 34** being located in a narrow crevice in close proximity to an anionic charge.

L3 ANSWER 16 OF 39 MEDLINE on STN

96229016. PubMed ID: 8686877. Dynamics of acrylodan-labeled bovine and human **serum albumin** entrapped in a sol-gel-derived biogel. Jordan J D; Dunbar R A; Bright F V. (Department of Chemistry, Natural Sciences and Mathematics Complex, State University of New York at Buffalo 14260-3000, USA.) Analytical chemistry, (1995 Jul 15) 67 (14) 2436-43. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB We investigate acrylodan-labeled bovine and human **serum albumin** (BSA-Ac and HSA-Ac) entrapped within a tetramethylorthosilane-derived biogel composite. The effects of biogel aging and drying were studied by following the acrylodan steady-state and time-resolved emission, the decay of anisotropy, and the dipolar relaxation kinetics as a function of

ambient storage time. The results indicate that there is a substantial amount of nanosecond and subnanosecond dipolar relaxation within the local environment surrounding **cysteine-34** in both proteins, even when they are fully encapsulated in a dry biogel. Time-resolved anisotropy experiments show that the acrylodan residue and the protein are able to undergo nanosecond motion within the biogel. The semiangle through which the acrylodan can process is the same for a freshly formed biogel and the native protein in buffer. However, once the biogel begins to dry, the semiangle increases (approximately 20 degrees and 10 degrees for BSA-Ac and HSA-Ac, respectively). This suggests that the "pocket" hosting the acrylodan reporter group opens as the biogel dries.

L3 ANSWER 17 OF 39 MEDLINE on STN

96062545. PubMed ID: 7586558. Calcium ion binding to clinically relevant chemical modifications of human **serum albumin**. Vorum H; Fisker K; Otagiri M; Pedersen A O; Kragh-Hansen U. (Department of Medical Biochemistry, University of Aarhus, Denmark.) Clinical chemistry, (1995 Nov) 41 (11) 1654-61. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB Calcium binding to glycated, penicilloylated, acetylated, and normal defatted human **serum albumin** as well as to mercapt- and nonmercaptalbumin was studied by equilibrium dialysis of radioactive Ca^{2+} . Binding was quantified by five Scatchard constants ($n_i = 1$, ($i = 1-4$) and $n_5 = 10$). Glycation resulted in increased k_1 - and k_2 -values and unchanged k_3 - k_5 -values, whereas penicilloylation increased all five association constants. The increments were greater the more pronounced the modification, and the enhancements caused by penicilloylation were, for the same degree of modification, greater than those produced by glycation. In contrast, acetylation by acetylsalicylate did not affect calcium binding. Likewise, binding to mercapt- and nonmercaptalbumin was the same, a finding showing that the thiol group of **cysteine 34** is not important for calcium binding. D-Glucose and penicillin G are known to react with lysine residues of albumin, and the enhancement of binding resulting from glycation or penicilloylation is probably brought about by unspecific electrostatic effects, possibly supplemented by conformational changes of the protein molecule. The relative importance of the three domains of human **serum albumin** for calcium binding is discussed.

L3 ANSWER 18 OF 39 MEDLINE on STN

95168613. PubMed ID: 7864387. Dynamics surrounding **Cys-34** in native, chemically denatured, and silica-adsorbed bovine **serum albumin**. Wang R; Sun S; Bekos E J; Bright F V. (Department of Chemistry, Natural Sciences and Mathematics Complex, State University of New York at Buffalo 14260-3000.) Analytical chemistry, (1995 Jan 1) 67 (1) 149-59. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB We report the steady-state and time-resolved fluorescence of 6-acryloyl(dimethylamino)naphthalene (acrylodan) covalently attached to **Cys-34** in bovine **serum albumin** (BSA). For this conceptually simple system, complicated fluorescence intensity and anisotropy decay kinetics are observed. The steady-state and time-resolved results demonstrate the presence of an excited-state reaction for the BSA-acrylodan system. Additional analysis shows that dipolar relaxation of the environment surrounding acrylodan within BSA is responsible for most of the observed time-dependent evolution of the emission spectrum. The effects of temperature, chemical denaturation, and protein adsorption to a bare silica substrate are also investigated. These results demonstrate the complexity of the changes within a protein/biorecognition element that affect the signal from a single fluorescent reporter group.

L3 ANSWER 19 OF 39 MEDLINE on STN

95017307. PubMed ID: 7931850. Conformational changes induced in bovine **serum albumin** by the photodynamic action of haematoporphyrin. Timmins G S; Davies M J. (Department of Chemistry, University of York, Heslington, UK.) Journal of photochemistry and photobiology. B, Biology, (1994 Jul) 24 (2) 117-22. Journal code: 8804966. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB The photodynamic action of haematoporphyrin upon bovine **serum albumin**, spin-labelled at the **cysteine-34** residue, has been shown to: (i) increase its susceptibility to proteolysis by chymotrypsin and trypsin, and (ii) increase its susceptibility to denaturation by urea. This is thought to be the result of conformational changes caused by the formation of protein radicals, although contributions from subsequent radical reactions of, for example, amino acids, may also take place. Such species have previously been shown, by EPR spin-trapping, to be formed in this system. Increased proteolytic susceptibility of non spin-labelled protein is also observed upon photolysis with haematoporphyrin, indicating that the changes observed in the spin-labelled protein also occur in the native form, and are not artefactual in nature. The significance of these

photochemically-induced conformational changes within proteins in the photodynamic therapy of tumours, and other protein-radical systems is discussed.

L3 ANSWER 20 OF 39 MEDLINE on STN

94207012. PubMed ID: 8155715. The kinetic studies on the intramolecular SH, S-S exchange reaction of bovine mercaptalbumin. Kuwata K; Era S; Sogami M. (Department of Physiology, School of Medicine, Gifu University, Japan.) *Biochimica et biophysica acta*, (1994 Apr 13) 1205 (2) 317-24. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Bovine mercaptalbumin (BMA) has 17 disulfide bonds and one SH group at **Cys-34** which catalyzes the intramolecular SH, S-S exchange reaction (N-A isomerization, molecular aging) in the alkaline region at low ionic strength, resulting in the formation of the aged form (A-form). The aging reaction was completely reversible and strongly affected by environmental factors, such as pH, temperature, ionic strength, Ca²⁺, nonbranched short-chain fatty acids, etc. Disulfide configuration (or pairing of disulfide bonds) was affected by the environmental factors. Obtained results might support the concept of Klotz (1966) that protein conformation (or three-dimensional structure) is dependent upon (i) the primary structure and (ii) constituents of the solvent.

L3 ANSWER 21 OF 39 MEDLINE on STN

93307207. PubMed ID: 8319609. Metabolism of the food-borne carcinogens 2-amino-3-methylimidazo-[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline in the rat as a model for human biomonitoring. Turesky R J; Stillwell W G; Skipper P L; Tannenbaum S R. (Nestec Ltd., Nestle Research Center, Lausanne, Switzerland.) *Environmental health perspectives*, (1993 Mar) 99 123-8. Journal code: 0330411. ISSN: 0091-6765. Pub. country: United States. Language: English.

AB Metabolism of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazol[4,5-f]quinoxaline (MeIQx) and their binding to blood proteins were examined in the rat to develop methods of human biomonitoring. Hemoglobin and **serum albumin** were among many blood proteins modified. Approximately 0.01% of the dose for both compounds was bound to these proteins, and induction of cytochrome P-450 with polychlorobiphenyls resulted in decreased levels of adduction. Hemoglobin sulfinic acid amide adducts could not be detected for either amine, however, as much as 10% of the IQ bound to albumin was characterized as an **N2-cysteine(34)sulfinyl-IQ** linkage. Human dosimetry of these carcinogens through such adducts may prove difficult due to the low levels of protein binding. Major routes of detoxification of both contaminants included cytochrome P-450-mediated ring hydroxylation at the C-5 position followed by conjugation to glucuronic or sulfuric acid. Direct conjugation to the exocyclic amine group through N-glucoronidation and sulfamate formation were other important routes of inactivation, but N-acetylation was a minor pathway. The N-glucoronide conjugate of the mutagenic metabolite N-hydroxy-MeIQx was also detected in urine. Rats given MeIQx at 10 micrograms/kg excreted 20% of the dose in urine within 24 hr and the remainder was recovered in feces. The N2-glucuronide was the major metabolite found in urine and accounted for 4% of the total dose. The other metabolites cited above also were excreted in urine at amounts ranging from 0.5 to 3% of the dose, whereas 0.5 to 2% was detected as unmetabolized MeIQx. (ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 22 OF 39 MEDLINE on STN

92366524. PubMed ID: 1502182. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of **serum albumin**. Stamler J S; Jaraki O; Osborne J; Simon D I; Keaney J; Vita J; Singel D; Valeri C R; Loscalzo J. (Department of Medicine, Harvard University, Cambridge, MA 02138.) *Proceedings of the National Academy of Sciences of the United States of America*, (1992 Aug 15) 89 (16) 7674-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have recently shown that nitric oxide or authentic endothelium-derived relaxing factor generated in a biologic system reacts in the presence of specific protein thiols to form S-nitrosoprotein derivatives that have endothelium-derived relaxing factor-like properties. The single free cysteine of **serum albumin, Cys-34**, is particularly reactive toward nitrogen oxides (most likely nitrosonium ion) under physiologic conditions, primarily because of its anomalously low pK; given its abundance in plasma, where it accounts for approximately 0.5 mM thiol, we hypothesized that this plasma protein serves as a reservoir for nitric oxide produced by the endothelial cell. To test this hypothesis, we developed a methodology, which involves UV photolytic cleavage of the S--NO bond before reaction with ozone for chemiluminescence detection, with which to measure free nitric oxide, S-nitrosothiols, and S-nitrosoproteins in biologic systems. We found that human plasma contains approximately 7 microM S-nitrosothiols, of which 96% are

S-nitrosoproteins, 82% of which is accounted for by S-nitroso-serum albumin. By contrast, plasma levels of free nitric oxide are only in the 3-nM range. In rabbits, plasma S-nitrosothiols are present at approximately 1 microm; 60 min after administration of NG-monomethyl-L-arginine at 50 mg/ml, a selective and potent inhibitor of nitric oxide synthetases, S-nitrosothiols decreased by approximately 40% (greater than 95% of which were accounted for by S-nitrosoproteins, and approximately 80% of which was S-nitroso-serum albumin); this decrease was accompanied by a concomitant increase in mean arterial blood pressure of 22%. These data suggest that naturally produced nitric oxide circulates in plasma primarily complexed in S-nitrosothiol species, principal among which is S-nitroso-serum albumin. This abundant, relatively long-lived adduct likely serves as a reservoir with which plasma levels of highly reactive, short-lived free nitric oxide can be regulated for the maintenance of vascular tone.

L3 ANSWER 23 OF 39 MEDLINE on STN

92240092. PubMed ID: 1368072. Immobilized metal ion affinity chromatography of serum albumins. Andersson L; Sulkowski E; Porath J. (Institute of Biochemistry and Biochemical Separation Center, Uppsala University, Sweden.) Bioseparation, (1991) 2 (1) 15-22. Journal code: 9011423. ISSN: 0923-179X. Pub. country: Netherlands. Language: English.

AB The interaction of several serum albumins with chelated (iminodiacetate, IDA) and immobilized (agarose-IDA) metal ions, Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺, was studied. There was no retention of human, bovine, porcine, murine and avian albumins on IDA-Zn(II) and IDA-Co(II) columns. However, all albumins studied, i.e., those of: man, cow, pig, dog, rabbit, rat, mouse, chicken and pigeon were retained on IDA-Cu(II) columns, and all except dog albumin were retained also on IDA-Ni(II). The recognition of albumins by chelated and immobilized transition metals seems to be related to an affinity for the imidazole side chains. It is postulated that one to three imidazoles is involved in this interaction, under the employed experimental conditions (pH 7.0; 1 M sodium chloride). There is no evidence for any significant contribution of tryptophan or cysteine (Cys 34) residues to the chromatographic event. The retention of defatted albumin and albumin oligomers (human), on IDA-Cu(II) columns was not significantly different from that of non-defatted albumin or albumin monomer, respectively.

L3 ANSWER 24 OF 39 MEDLINE on STN

92191338. PubMed ID: 1799947

0.70, 0.12 and 0.62, 0.18, respectively (Era et al. (1990]). However, there were no changes in f_{α} and f_{β} of the iodoacetamide-blocked A-form (IA-A-form) over the pH range from 5.5 to 9.1 in the absence of added salt or in 0.10 M KCl (f_{α} approximately 0.60, f_{β} approximately 0.20), indicating that the secondary structure of the IA-A-form might be similar to that of non-aged IA-BPA at pH 9.0 (B-form) in the absence of added salt, that is, the frozen B-form, stabilized covalently by the repairing of disulfide bonds. 2) The rigidity of the A- and IA-A-forms, as monitored by cross-relaxation times between irradiated and observed protein protons, was similar to or slightly higher than that of non-aged IA-BPA or BMA. (ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 26 OF 39 MEDLINE on STN

92003197. PubMed ID: 1912331. Carbamoylation of peptides and proteins in vitro by S-(N-methylcarbamoyl)glutathione and S-(N-methylcarbamoyl)cysteine, two electrophilic S-linked conjugates of methyl isocyanate. Pearson P G; Slatter J G; Rashed M S; Han D H; Baillie T A. (Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle 98195.) Chemical research in toxicology, (1991 Jul-Aug) 4 (4) 436-44. Journal code: 8807448. ISSN: 0893-228X. Pub. country: United States. Language: English.

AB The reactivity toward peptides and proteins of S-(N-methylcarbamoyl)glutathione (SMG), the glutathione conjugate of methyl isocyanate, and the corresponding cysteine adduct, S-(N-methylcarbamoyl)cysteine (SMC), was investigated with the aid of in vitro model systems. Incubation of SMC or a trideuteriomethyl analogue of SMC with either the reduced or oxidized forms of oxytocin afforded similar mixtures of mono-, bis- and tris-N-methylcarbamoylated peptides. Structure elucidation of the mono and bis adducts by fast atom bombardment tandem mass spectrometry indicated that carbamoylation of oxytocin occurred preferentially at Cys-6 and that Cys-1 and/or Tyr-2 were secondary sites of modification. Upon incubation of S-(N-([14C]methyl)carbamoyl)glutathione (14C-SMG) with native bovine **serum albumin** (BSA), radioactivity became bound covalently to the protein in a time- and concentration-dependent fashion. "Blocking" of the lone **Cys-34** thiol group of BSA in the form of a disulfide prior to exposure of the protein to 14C-SMG failed to decrease significantly the extent or time course of this covalent binding. It is concluded that carbamate thioester conjugates of MIC are reactive, carbamoylating entities which can donate the elements of MIC

the BSA-bound fraction of the ligand did not change with ligand/protein ratio. MMC strongly and stoichiometrically bound to mercaptalbumin even at a molar ratio of 1:1. In contrast, the albumin bound fractions of three other MM ligands increased with concomitant decrease in ligand/protein ratio and with time except for the alkylated albumin, the highest binding being shown by mercaptalbumin. Binding of S-2-nitrophenyl-glutathione, a GSH analog with a hydrophobic S-substituent, to albumin species occurred similarly to that of GS-MM. However, GSH and oxidized glutathione (GSSG) interacted differently with albumin; mercaptalbumin showed the lowest affinity for GSH, and GSSG scarcely interacted with all BSA species. These results suggest that the sulfhydryl group at **Cys-34** is not the only site of BSA that interacts with MM compounds and that albumin interacts preferentially with the hydrophobic domains of a mercurial ligand rather than its hydrophilic peptide moiety.

L3 ANSWER 29 OF 39 MEDLINE on STN

90216119. PubMed ID: 2323880. Structural transition of bovine plasma albumin in the alkaline region--the N-B transition. Era S; Itoh K B; Sogami M; Kuwata K; Iwama T; Yamada H; Watari H. (Department of Physiology, School of Medicine, Gifu University, Japan.) International journal of peptide and protein research, (1990 Jan) 35 (1) 1-11. Journal code: 0330420. ISSN: 0367-8377. Pub. country: Denmark. Language: English.

AB Bovine plasma albumin (BPA) has approximately one SH group (**Cys-34**) which catalyzes the intramolecular SH, S-S exchange reaction in the alkaline region at low ionic strength, resulting in the formation of the aged form. So, the N-B transition at ionic strength above 0.20 and below 0.10 was studied using BPA and iodoacetamide-blocked BPA (IA-BPA), respectively. (1) pH profiles of $[\theta]_{262}$ and $[\theta]_{268}$ of BPA in 0.20 M KCl showed the characteristic changes in the pH region 7.0-9.0, corresponding to the N-B transition. On going from pH 7.0 to 9.0 in 0.10 M KCl or NaCl, IA-BPA did not show significant changes in rotational relaxation times of tryptophyl fluorophors, CD-resolved secondary structures, spin-echo 1H-n.m.r. spectra and cross-relaxation times (TIS) between irradiated and observed protein protons, which might reflect the rigidity of the domains and/or subdomains. On the other hand, rotational relaxation times of 1-anilino-8-naphthalenesulfonate-IA-BPA complex (IA-BPA-ANS 0.9, molar ratio of ANS to IA-BPA = 0.9/1) showed significant decreases from 131 to 114 ns on going from the N- to the B-forms in 0.10 M KCl. The above results and reported experimental evidence might indicate that on going from the N- to the B-forms in 0.10 M KCl or NaCl, the mutual movement of subdomains, connected with a flexible hinge region (Brown & Shockley (

covalent adduct formed in vitro between acetaminophen and bovine **serum albumin**. Hoffmann K J; Streeter A J; Axworthy D B; Baillie T A. *Chemico-biological interactions*, (1985 Feb-Apr) 53 (1-2) 155-72. Journal code: 0227276. ISSN: 0009-2797. Pub. country: Netherlands. Language: English.

AB The structure of the covalent adduct formed in vitro between [¹⁴C]-acetaminophen ([¹⁴C]APAP) and bovine **serum albumin** (BSA) has been investigated with the aid of new analytical methodology. The APAP-BSA adduct, isolated from mouse liver microsomal incubations to which the radiolabeled drug and BSA had been added, was cleaved using a combination of specific (cyanogen bromide) and non-specific (acid hydrolysis) procedures, following which the mixture of amino acids obtained was derivatized, in aqueous solution, with ethyl chloroformate. The resulting ethoxycarbonyl derivatives were recovered by extraction into ethylacetate, methylated and subjected to profile analysis using both reverse-phase and normal-phase HPLC techniques. In each HPLC step, one major radioactive amino acid adduct was detected and was identified by mass spectrometry as the derivative of 3-cystein-S-yl-4-hydroxyaniline. Based on this finding, and with a knowledge of the behavior under acidic hydrolysis conditions of the 3-cysteinyl conjugate of APAP, it could be concluded that the major APAP-BSA adduct is one in which the drug is bound, via a thioether linkage at the C-3 position, to a sulfhydryl group on the protein. Furthermore, it could be established that this -SH function almost certainly is that associated with the **cys-34** residue of BSA.

L3 ANSWER 35 OF 39 MEDLINE on STN

83231442. PubMed ID: 6860638. Resonance energy transfer between **cysteine-34**, tryptophan-214, and tyrosine-411 of human **serum albumin**. Hagag N; Birnbaum E R; Darnall D W. *Biochemistry*, (1983 May 10) 22 (10) 2420-7. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Reaction of p-nitrophenyl anthranilate with human **serum albumin** at pH 8.0 results in esterification of a single anthraniloyl moiety with the hydroxyl group of tyrosine-411. The absorption spectrum of the anthraniloyl group overlaps the fluorescence emission of the single tryptophan residue at position 214. This study complements that of the preceding paper [Suzukida, M., Le, H. P., Shahid, F., McPherson, R. A., Birnbaum, E.R., & Darnall, D. W. (1983) *Biochemistry* (preceding paper in this issue)] where an azomercurial group was introduced at **cysteine-34**. Anthraniloyl fluorescence was also quenched by the az

L3 ANSWER 38 OF 39 MEDLINE on STN

82239366. PubMed ID: 7096338. The biosynthesis of rat **serum albumin**.
In vivo studies on the formation of the disulfide bonds. Peters T Jr;
Davidson L K. Journal of biological chemistry, (1982 Aug 10) 257 (15)
8847-53. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United
States. Language: English.

AB In order to learn at what stage the disulfide bonds of albumin are formed
during its biosynthesis, we perfused rat livers with iodoacetamide and
then isolated the intracellular precursor, proalbumin, from organelles
known to be in the pathway of albumin synthesis and secretion. The
alkylated cysteines in proalbumin were determined as a measure of its
thiol groups in vivo. Proalbumin of smooth microsomes was found to
contain a single thiol, which is proposed to be the noncoupling cysteine
occurring residue 34 in circulating albumin. Proalbumin in rough
microsomes contained an average of two cysteines; the additional cysteine
thiol was largely situated in the COOH-terminal region and disappeared
rapidly after blocking albumin synthesis with cycloheximide. In nascent
chains of proalbumin, about 45% of the cysteine + cystine was in the thiol
form. From these findings we propose that disulfide bond formation begins
while the nascent chain is still attached to the ribosome and proceeds in
an NH2 to COOH direction. The disulfide bonding apparently is completed
into the endoplasmic reticulum. Possible intermediates in the process
such as mixed disulfide forms of proalbumin with glutathione or cystamine
were not detected. We suggest that **cysteine-34** does not participate
in disulfide bonding because the NH2 terminus of proalbumin remains
loosely bound to the membrane, attached by a hydrophobic segment of the
chain at residues 21-27.

L3 ANSWER 39 OF 39 MEDLINE on STN

80156821. PubMed ID: 6244951. Temperature behaviour of human **serum
albumin**. Wetzel R; Becker M; Behlke J; Billwitz H; Bohm S; Ebert B;
Hamann H; Krumbiegel J; Lassmann G. European journal of biochemistry /
FEBS, (1980 Mar) 104 (2) 469-78. Journal code: 0107600. ISSN:
0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of.
Language: English.

AB Structural alterations of albumin, their dependence on concentration and
the role of free --SH groups at thermal denaturation, as well as the
reversibility of thermally induced structural changes, were studied.
Application of various physical methods provides information on a series
of structural parameters in a major concentration range. Apart from
changes of the helix content, heat treatment gives rise to beta structures
which are amplified on cooling and which are correlated with the
aggregation of albumin. With rising temperature and concentration the
proportion of beta structures and aggregates increases. At degrees of
denaturation of up to 20% complete renaturation is possibly in every case.
The structure content is concentration-dependent even at room temperature.
It may be that intermolecular interactions induce additional alpha-helix
structures which are less stable, however, than the ones stabilized by
intramolecular interactions. Unfolding of the pocket containing the free
--SH group of **cysteine-34** enables disulphide bridges to be formed
leading to stable aggregates and irreversible structural alterations.
Through binding of N-ethylmaleimide to free --SH groups, which blocks the
formation of disulphide bridges, it is possible to prevent aggregation and
irreversible conformational changes. At temperatures below 65--70 degrees
C, oligomers are formed mainly via intermolecular beta structures.

=> d his

(FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005

L1 68146 S (SERUM ALBUMIN)
L2 74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3 39 S L2 AND PY<2000

=> s l1 and pharmacolog?
1979072 PHARMACOLOG?

L4 15041 L1 AND PHARMACOLOG?

=> s l4 and (conjugat? or fusion or linked)
78444 CONJUGAT?
125944 FUSION
247085 LINKED

L5 1533 L4 AND (CONJUGAT? OR FUSION OR LINKED)

=> s l5 (increased stability or increased clearance or increased half-life)

MISSING OPERATOR 'L5 (INCREASED'

The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s 15 and (increased stability or delayed clearance or increased circulation or increased half-life)

1214534 INCREASED
134740 STABILITY
1109 INCREASED STABILITY
(INCREASED(W)STABILITY)
154790 DELAYED
90311 CLEARANCE
332 DELAYED CLEARANCE
(DELAYED(W)CLEARANCE)
1214534 INCREASED
198277 CIRCULATION
60 INCREASED CIRCULATION
(INCREASED(W)CIRCULATION)
1214534 INCREASED
226783 HALF
398797 LIFE
133 INCREASED HALF-LIFE
(INCREASED(W)HALF(W)LIFE)

L6 2 L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED CIRCULATION OR INCREASED HALF-LIFE)

=> d 16,cbib,ab,1-2

L6 ANSWER 1 OF 2 MEDLINE on STN

2001506683. PubMed ID: 11555696. Prolonged in vivo anticoagulant activity of a hirudin-albumin **fusion** protein secreted from *Pichia pastoris*. Sheffield W P; Smith I J; Syed S; Bhakta V. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ont., Canada.. sheffiel@mcmaster.ca) . Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, (2001 Sep) 12 (6) 433-43. Journal code: 9102551. ISSN: 0957-5235. Pub. country: England: United Kingdom. Language: English.

AB Hirudin is a small, proteinaceous thrombin inhibitor that clears rapidly from the circulation. A hexahistidine-tagged hirudin-rabbit **serum albumin** (RSA) **fusion** protein, HLAH6, was characterized following secretion from *Pichia pastoris*. HLAH6 bound to immobilized nickel, anti-RSA, and anti-hexahistidine antibodies, and contained the expected (ITYTD) N-terminus. Its spectrometric mass was 74,490 (versus the theoretical mass of 74,410 and sodium dodecyl sulfate-polyacrylamide gel electrophoresis mobility of 84 kDa). The terminal catabolic half-life in rabbits of HLAH6, recombinant *Pichia*-derived His-tagged RSA, or plasma-derived RSA did not differ. Injection of 2 mg/kg HLAH6 into rabbits raised the activated partial thromboplastin time (aPTT) above initial values for 4-24 h, while the equimolar dose of unfused hirudin was without significant effect. A higher dose of HLAH6 (3 mg/kg functional HLAH6, equivalent to 37.6 thrombin-inhibitory units/g) raised the aPTT by 2.0- to 2.5-fold; the elevation persisted for > 48 h. Importantly, both HLAH6 and unfused hirudin inhibited clot-bound thrombin. Our results suggest that HLAH6 exhibits not only **delayed clearance**, but also prolonged biological activity in vivo compared with unfused hirudin.

L6 ANSWER 2 OF 2 MEDLINE on STN

90167655. PubMed ID: 2306723. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-**conjugated** poly(ethylene glycol)-poly(aspartic acid) block copolymer. Yokoyama M; Miyauchi M; Yamada N; Okano T; Sakurai Y; Kataoka K; Inoue S. (Institute of Biomedical Engineering, Tokyo Women's Medical College, Japan.) Cancer research, (1990 Mar 15) 50 (6) 1693-700. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Adriamycin (ADR), an anthracycline anticancer drug, was bound to the poly(aspartic acid) chain of poly(ethylene glycol)-poly(aspartic acid) block copolymer by amide bond formation between an amino group of Adriamycin and the carboxyl groups of the poly(aspartic acid) chain. The polymeric drug thus obtained was observed to form a micelle structure possessing diameter of approximately 50 nm, with a narrow distribution, in phosphate-buffered saline and to show excellent water solubility despite a large amount of ADR introduction. Further, it was able to be stored in lyophilized form without losing its water solubility in the redissolving procedure. **Increased stability** of the bound Adriamycin molecules in phosphate-buffered saline and elimination of binding affinity for bovine **serum albumin** due to the micelle formation were further advantages of this polymeric drug. In vivo high anticancer activity of this micelle-forming polymeric drug against P 388 mouse leukemia was obtained with less body weight loss than that seen with free ADR, due to low toxicity as compared with free ADR.

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(FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005

L1 68146 S (SERUM ALBUMIN)
L2 74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3 39 S L2 AND PY<2000
L4 15041 S L1 AND PHARMACOLOG?
L5 1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6 2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED

=> s 14 and (maleimid?)

4746 MALEIMID?
L7 47 L4 AND (MALEIMID?)

=> s 17 and py<2000

12397709 PY<2000
L8 28 L7 AND PY<2000

=> d 18,cbib,ab,1-28

L8 ANSWER 1 OF 28 MEDLINE on STN

1999261936. PubMed ID: 10330050. Glycated albumin stimulation of PKC-beta activity is linked to increased collagen IV in mesangial cells. Cohen M P; Ziyadeh F N; Lautenslager G T; Cohen J A; Shearman C W. (Institute of Metabolic Research and Exocell, University City Science Center, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) American journal of physiology, (1999 May) 276 (5 Pt 2) F684-90. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB Albumin modified by Amadori-glucose adducts induces coordinate increases in the expression of extracellular matrix proteins, transforming growth factor (TGF)-beta1, and the TGF-beta type II receptor in glomerular mesangial cells. Because activation of protein kinase C (PKC) accompanies the increased mesangial cell expression of matrix proteins and TGF-beta1 induced by high ambient glucose, we postulated that glycated albumin (GA) modulates PKC activity and that PKC participates in mediating the GA-induced stimulation of matrix production. To test this hypothesis, we examined the effects of PKC inhibitors on collagen type IV production by mouse or rat mesangial cells incubated with GA, and the influence of GA on PKC activity in these cells. Increased collagen type IV production evoked by GA in 5.5 and 25 mM glucose in mouse mesangial cells was prevented by both general (GF-109203X) and beta-specific (LY-379196) PKC inhibitors. Total PKC activity, measured by phosphorylation of a PKC-specific substrate, increased with time after exposure of rat mesangial cells to GA compared with the nonglycated, glucose-free counterpart. GA caused an increase in PKC-beta1 membrane-bound fraction and in total PKC activity in media containing physiological (5.5 mM) glucose concentrations in rat mesangial cells, confirming that the glucose-modified protein, and not a "hyperglycemic" milieu, was responsible. The findings indicate that Amadori-modified albumin stimulates mesangial cell PKC activity, and that activation of the PKC-beta isoform is linked to the stimulation of collagen type IV production.

L8 ANSWER 2 OF 28 MEDLINE on STN

1999008560. PubMed ID: 9794429. Inhibition by dexamethasone of antigen-induced c-Jun N-terminal kinase activation in rat basophilic leukemia cells. Hirasawa N; Sato Y; Fujita Y; Mue S; Ohuchi K. (Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Sendai, Japan.. hirasawa@mail.pharm.tohoku.ac.jp) . Journal of immunology (Baltimore, Md. : 1950), (1998 Nov 1) 161 (9) 4939-43. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.

AB Antigen stimulation of IgE-sensitized rat basophilic leukemia RBL-2H3 cells induced activation of c-Jun N-terminal kinase (JNK) within a few minutes with maximum activity attained 40 min later. The increase in JNK activity was accompanied with an increase in phosphorylation of c-Jun in the cells. The Ag-induced JNK activation was inhibited by the phosphatidylinositol 3-kinase inhibitors wortmannin (10-100 nM) and LY 294002 (100 microM) but not by the protein kinase C inhibitors calphostin C (1 and 3 microM) and Ro 31-8425 (1 and 3 microM). Pretreatment with dexamethasone (10 and 100 nM) for 18 h inhibited the Ag-induced increase in JNK activity in a concentration-dependent manner. At least 6 h of preincubation with dexamethasone was necessary to inhibit the Ag-induced JNK activation. The phosphorylation of c-Jun induced by the Ag stimulation was reduced by pretreatment with dexamethasone without reduction of the content of c-Jun protein. The Ag-induced activation of

the JNK kinase kinase mitogen-activated protein kinase-extracellular signal-regulated kinase kinase-1 was also inhibited by pretreatment with dexamethasone at 10 and 100 nM. These findings indicate that dexamethasone reduces JNK protein level and inhibits the Ag-induced activation of JNK resulting in the inhibition of c-Jun phosphorylation.

L8 ANSWER 3 OF 28 MEDLINE on STN

1998185800. PubMed ID: 9525088. Albumin conjugates of the anticancer drug chlorambucil: synthesis, characterization, and in vitro efficacy. Kratz F; Beyer U; Roth T; Schutte M T; Unold A; Fiebig H H; Unger C. (Tumor Biology Center, Dept. Med. Oncology, Freiburg, Germany.) Archiv der Pharmazie, (1998 Feb) 331 (2) 47-53. Journal code: 0330167. ISSN: 0365-6233. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB In our efforts to improve the selectivity and toxicity profile of antitumor agents, four **maleimide** derivatives of chlorambucil (1-4) were bound to thiolated human **serum albumin** which differ in the stability of the chemical link between drug and spacer. 1 is an aliphatic **maleimide** ester derivative of chlorambucil, whereas 2-4 are acetaldehyde, acetophenone, and benzaldehyde carboxylic hydrazone derivatives. HPLC stability studies at pH 5.0 with the related model compounds 5, 7, 8, and 9, in which chlorambucil was substituted by 4-phenylbutyric acid, demonstrated that the carboxylic hydrazone derivatives have acid-sensitive properties; the acid lability of 7 was particularly prominent with a half-life of only a few hours. The alkylating activity of albumin-bound chlorambucil was determined with the aid of 4-(4-nitrobenzyl)-pyridine (NBP), demonstrating that on average three equivalents were protein-bound. Evaluation of the cytotoxicity of free chlorambucil and the respective albumin conjugates in the MCF7 mammary carcinoma and MOLT4 leukemia cell line employing a propidium iodide fluorescence assay demonstrated that the conjugate in which chlorambucil was bound to albumin through an ester bond was not as active as chlorambucil. In contrast, the conjugates in which chlorambucil was bound to albumin through carboxylic hydrazone bonds were as or more active than chlorambucil in both cell lines. In particular, the conjugate in which chlorambucil was bound to albumin through an acetaldehyde carboxylic hydrazone bond exhibited IC50 values which were approximately 4-fold (MCF7) to 13-fold (MOLT4) lower than those of chlorambucil. Preliminary toxicity studies in mice showed that this conjugate can be administered at higher doses in comparison to unbound chlorambucil.

L8 ANSWER 4 OF 28 MEDLINE on STN

1998136036. PubMed ID: 9477169. Preparation, characterization and in vitro efficacy of albumin conjugates of doxorubicin. Kratz F; Beyer U; Collery P; Lechenault F

phosphorylation of vinculin and that this effect is involved in the early loss of endothelial barrier function. Vinculin localization and phosphorylation, PKC activity, and albumin permeability were studied in cultured coronary endothelial monolayers from rats. Ten minutes after the onset of metabolic inhibition by 5 mM potassium cyanide and 5 mM 2-deoxy-D-glucose, immunofluorescence of vinculin at cell-to-cell and cell-to-matrix contacts faded, whereas total cellular vinculin content remained unchanged. During the same time period, vinculin phosphorylation at tyrosine and serine sites increased by 3.9- and 3.5-fold, respectively. Vinculin phosphorylation was related to activation of PKC and an unidentified tyrosine kinase and was elicited by a rise in cytosolic Ca²⁺ within energy-depleted endothelial cells. Conditions inhibiting vinculin phosphorylation also reduced monolayer permeability induced by energy depletion. These data indicate that vinculin phosphorylation is involved in the progression of hyperpermeability during energy depletion in coronary endothelial monolayers.

L8 ANSWER 6 OF 28 MEDLINE on STN

97361240. PubMed ID: 9218134. Negative regulation of MAP kinase by diacylglycerol-dependent mechanisms via G protein-coupled receptors in rat basophilic RBL-2H3 (ml) cells. Hirasawa N; Mue S; Ohuchi K. (Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University Sendai, Miyagi, Japan.) Cellular signalling, (1997 May-Jun) 9 (3-4) 319-22. Journal code: 8904683. ISSN: 0898-6568. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Carbachol and 5'-(N-ethylcarboxamido)-adenosine (NECA), stimulants of G protein-coupled receptors, induce MAP kinase activation in the muscarinic ml receptor-transfected mast cell line, RBL-2H3 (ml) cells. The phospholipase C inhibitor neomycin and the phosphatidate phosphohydrolase inhibitor propranolol augmented MAP kinase activation induced by carbachol and NECA without affecting the antigen-induced MAP kinase activation. Furthermore, the duration of MAP kinase activation induced by carbachol or NECA was also prolonged by neomycin and propranolol. The specific protein kinase C inhibitor Ro 31-8425 enhanced the carbachol- or NECA-induced MAP kinase activation. These findings suggest that the MAP kinase activation mediated by the G protein-coupled receptors is negatively regulated by diacylglycerol and activated protein kinase C(s).

L8 ANSWER 7 OF 28 MEDLINE on STN

97302612. PubMed ID: 9158859. Covalent linkage of recombinant hirudin to poly(ethylene terephthalate) (Dacron): creation of a novel antithrombin surface. Phaneuf M D; Berceci S A; Bide M J; Quist W C; LoGerfo F W. (Deaconess Hospital/Harvard Medical School

bound to a clinically utilized biomaterial (Dacron) while still maintaining its ability to bind and inhibit thrombin.

L8 ANSWER 8 OF 28 MEDLINE on STN

97102715. PubMed ID: 8946955. Quantitative analysis of exocytosis visualized by a video-enhanced light/fluorescence microscope reveals two distinct components of exocytosis in RBL-2H3 cells. Ozawa K; Kobayashi H; Kawai E; Suzuki E; Nonomura Y; Masujima T. (Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Japan.) FEBS letters, (1996 Nov 25) 398 (1) 67-73. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Rat basophilic leukemia (RBL-2H3) cells, which exhibit Ca²⁺-dependent secretion of granules when stimulated with antigen or the Ca²⁺-ionophore A23187, were observed under a video-enhanced light/fluorescence microscope. Exocytotic events of individual granules were visualized in individual cells stimulated with antigen or A23187. Exocytosis of granules stimulated with A23187 showed two peaks in the time course. The earlier one was inhibited by selective inhibitors of protein kinase C (Ro31-8425, Ro31-8220, and chelerythrine) and the other was inhibited by an inhibitor of phosphatidate hydrolase, propranolol. Exocytosis by antigen stimulation, however, showed only one peak, which was inhibited by the selective inhibitors of protein kinase C, but not by propranolol. These results indicate that at least two distinct components of exocytosis exist in RBL-2H3 cells.

L8 ANSWER 9 OF 28 MEDLINE on STN

97027829. PubMed ID: 9125277. Interaction of glucose and metformin with isolated red cell membrane. Freisleben H J; Furstenberger H J; Deisinger S; Freisleben K B; Wiernsperger N; Zimmer G. (Gustav-Embden-Zentrum der Biologischen Chemie, Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt/Main, Germany.) Arzneimittel-Forschung, (1996 Aug) 46 (8) 773-8. Journal code: 0372660. ISSN: 0004-4172. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Isolated human erythrocyte membranes (red blood cell (RBC) ghosts) were incubated with glucose at 5, 10, 20 and 100 mmol/l concentrations, with insulin (0.01 to 200 mU/l) and metformin (CAS 657-24-9) 0.5 up to 50.0 mmol/l). Binding studies with ¹⁴C-glucose and subsequent gel electrophoresis revealed 60% of the radioactivity around

IRGERA as a model peptide, we found that all six liposomal constructs strongly elicited the production of anti-peptide IgG antibodies. This immune response was therefore independent of the length of the linkers (ranging between 0.3 and 1.6 nm) and of the nature of the linkage. between the peptide and the thiol-reactive moieties of the cross-linkers, i.e. stable thioether or bio-reducible disulfide bonds.

L8 ANSWER 11 OF 28 MEDLINE on STN

96174859. PubMed ID: 8593275. Preparation and characterization of conjugates of (modified) human **serum albumin** and liposomes: drug carriers with an intrinsic anti-HIV activity. Kamps J A; Swart P J; Morselt H W; Pauwels R; De Bethune M P; De Clercq E; Meijer D K; Scherphof G L. (Groningen Institute for Drug Studies, Department of Physiological Chemistry, Groningen University, Groningen, The Netherlands.) *Biochimica et biophysica acta*, (1996 Jan 31) 1278 (2) 183-90. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Human **serum albumin** (HSA) derivatized with cis-aconitic anhydride (Aco-HSA) that was earlier shown to inhibit replication of human immunodeficiency virus type 1 (HIV-1), was covalently coupled to conventional liposomes, consisting of phosphatidylcholine, cholesterol and **maleimido-4-(p-phenylbutyryl)phosphatidylethanolamine**, using the heterobifunctional reagent N-succinimidyl-S-acetylthioacetate (SATA). The amount of HSA that could be coupled to the liposomes depended on derivatization of the HSA and ranged from 64.2 +/- microgram HSA/micromol total lipid for native HSA to 29.5 +/- 2.7 microgram HSA/micromol total lipid for HSA in which 53 of the epsilon amino groups of lysine were derivatized with cis-aconitic anhydride (Aco53-HSA). Incorporation of 3.8 mol% of total lipid of a poly(ethylene glycol) derivative of phosphatidylethanolamine (PEG-PE) in the liposomes resulted in a lower coupling efficiency of Aco-HSA. The elimination and distribution of the liposomal conjugates in rats in vivo was largely dependent on the modification of the HSA coupled to the liposomes. With native HSA-liposomes, more than 70% of the conjugate was still found in the blood plasma 30 min after i.v. injection in rats, while at this time Aco-HSA-liposomes were completely cleared from the circulation. The rapid clearance of conventional Aco-HSA-liposomes was due to a rapid uptake into the liver and could be considerably decreased by incorporating PEG-PE in the liposomal bilayer. After 3 h 60% of Aco-HSA-PEG-liposome conjugates were found in the blood. In an in vitro anti-HIV-1 assay, the 50% inhibitory concentrations (IC50) for Aco39-HSA-lip

endothelial cells and BSA-coated plastic. However, the responses to C5a were unaffected by Ro 31-8425. These results suggest that, although activation of PKC can promote up-regulation of Mac-1 and adhesion of neutrophils, this does not appear to be the physiological pathway. A non-selective protein kinase inhibitor, staurosporine, inhibited both PBu2 and C5a-stimulated adhesion. This suggests that a protein kinase other than PKC, possibly a tyrosine protein kinase, is likely to be involved in mediating C5a-stimulated Mac-1 up-regulation and adhesion. These results emphasise the need for caution in interpreting experiments and assuming a role for PKC. Use of a potent and selective inhibitor of PKC, Ro 31-8425, provides more definitive information.

L8 ANSWER 13 OF 28 MEDLINE on STN

94012853. PubMed ID: 8408112. Detection of remnant proteolytic activities in unimplanted glutaraldehyde-treated bovine pericardium and explanted cardiac bioprostheses. Simionescu D; Simionescu A; Deac R. (Cardiovascular Surgery Research Department, Public Health and Medical Research Institute, Tirgu Mures, Romania.) Journal of biomedical materials research, (1993 Jun) 27 (6) 821-9. Journal code: 0112726. ISSN: 0021-9304. Pub. country: United States. Language: English.

AB The presence and activity of proteolytic enzymes has been investigated in vitro on soluble and insoluble preparations obtained from both unimplanted and implanted glutaraldehyde-treated bovine parietal pericardium. Using detection by colorimetric techniques, soluble preparations were shown to hydrolyze enzyme substrates that are characteristic for trypsin-like proteases, cathepsin-like proteases, and collagenase. As detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in gradient gels and gel filtration on Sepharose CL-6B, insoluble (pellet) preparations degraded denatured type I collagen in a time-dependent pattern, producing low-molecular-weight fragments. These activities were partially inhibited by phenylmethylsulfonyl fluoride, N-ethyl maleimide, soybean trypsin inhibitor, para-chloromercuribenzoic acid, or ethylenediaminetetraacetic acid, suggesting the presence of a heterogeneous enzymatic mixture. Insoluble preparations incubated with pure pericardial dermatan sulfate proteoglycan detached the glycosaminoglycan chains from their core protein carrier, producing a digestion pattern similar to Cathepsin C. These findings demonstrate the presence of active proteases in glutaraldehyde-fixed bovine pericardium per se and in explanted pericardial bioprosthetic cardiac valves, an additional factor that might contribute to intrinsic extracellular matrix degeneration in pericardial bioprosthetic devices.

L8 ANSWER 14 OF 28 MEDLINE on STN

93119500. PubMed ID: 1476657. Immunomodulating activity of 1,2-difattyacyl-3-mercaptoglycerol adducts. Gemeiner M; Leidinger E; Miller I; Moroder L. (Institut für Medizin

conjugated with mercaptosuccinyl bovine **serum albumin** (MS.BSA) using N-[beta-(4-diazophenyl)ethyl]**maleimide** (DPEM) as a heterobifunctional coupling agent. An enzyme marker was similarly prepared by coupling EP with beta-D-galactosidase (beta-Gal; EC 3.2.23) via DPEM. This ELISA was specific for EP and showed a very slight cross-reactivity with its major metabolite, cis-hydroxy acid of EP (0.91%), but none with 4'-demethylepipodophyllotoxin and drugs commonly used with EP in combination chemotherapy for cancer treatment. The values for EP concentration detected by this assay were comparable with those detected by the high-performance liquid chromatography (HPLC) method. However, the ELISA was about 1,250 times more sensitive in detecting EP at lower concentrations. Using this assay, drug levels were easily determined in the blood and urine of rats for 7 h after i.v. administration of EP at a single dose of 3 mg/kg. Due to its sensitivity and specificity for EP, the ELISA should prove to be a valuable new tool for use in clinical **pharmacological** studies.

L8 ANSWER 16 OF 28 MEDLINE on STN

90001218. PubMed ID: 2790016. Synthesis of a superoxide dismutase derivative that circulates bound to albumin and accumulates in tissues whose pH is decreased. Inoue M; Ebashi I; Watanabe N; Morino Y. (Department of Biochemistry, Kumamoto University Medical School, Japan.) **Biochemistry**, (1989 Aug 8) 28 (16) 6619-24. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Protection of tissues from oxidative stress is one of the major prerequisites for aerobic life. Since intravenously injected Cu²⁺/Zn²⁺-type superoxide dismutase (SOD) disappears from the circulation with a short half-life of 5 min, its clinical use as a scavenger for superoxide radical is limited. We synthesized a human erythrocyte type SOD derivative (SM-SOD) by linking 2 mol of hydrophobic organic anion, alpha-4-[(6-(N-**maleimido**)hexanoyloxymethyl]cumyl]half-butyl-esterified poly(styrene-co-maleic acid) (SM), to the cysteinyl residues of the dimeric enzyme without decreasing enzymic activity. SM-SOD, but not SOD, bound to an albumin-Sepharose column; the bound SM-SOD was eluted by a buffer solution containing 0.5% sodium dodecyl sulfate or 10 mM warfarin, suggesting that SM-SOD reversibly binds to the warfarin site on albumin. Due to the amphipathic nature of the SMI moiety, SM-SOD bound also to cell membranes particularly when the pH was decreased. In vivo analysis in the rat revealed that intravenously injected SM-SOD circulated bound to albumin with a half-life of 6 h. Postischemic reperfusion arrhythmias were almost completely prevented by a single dose of SM-SOD, but not SOD. Thus, the prolonged half-life of SM-SOD in the circulation and its prefer

- Cancer research, (1987 Feb 15) 47 (4) 1076-80. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.
- AB In studies on antitumor antibody:drug conjugates as potential antitumor agents, methotrexate (MTX) was conjugated with a murine monoclonal antibody (aMM46) to an antigen on ascitic mouse mammary tumor MM46 cells (MM antigen) with human **serum albumin** (HSA) as an intermediary. MTX was linked to HSA which had been conditioned to have about 1 mol of thiol group per mol of HSA by dithiothreitol treatment followed by oxidation on standing at 4 degrees C. The MTX linking was performed, without protection of the thiol group of HSA, by using MTX N-succinimidyl ester prepared via MTX intramolecular anhydride. The resulting HSA:MTX was reacted with the immunoglobulin with the **maleimide** group introduced. The aMM46:HSA:MTX obtained retained both antibody binding and drug activities. The cytotoxicity of aMM46:HSA:MTX against MM antigen-positive MM46 cells was greater than that of control 96.5 (anti-human melanoma-associated antigen, p97):HSA:MTX and was inhibited by unconjugated aMM46. No different cytotoxicity of aMM46:HSA:MTX compared with that of 96.5:HSA:MTX was observed against MM antigen-negative mouse mammary tumor MM48 cells. The presence of ammonium chloride or leupeptin abrogated the selective cytotoxicity against MM46 cells of aMM46 conjugate but did not affect the nonspecific cytotoxicity of 96.5:HSA:MTX. These results support the idea that the selective cytotoxicity of aMM46:HSA:MTX is antibody directed and exhibited through lysosomal degradation of the conjugate.
- L8 ANSWER 19 OF 28 MEDLINE on STN
86026321. PubMed ID: 2996599. ESR spectral changes induced by chlorpromazine in spin-labeled erythrocyte ghost membranes. Yamaguchi T; Watanabe S; Kimoto E. Biochimica et biophysica acta, (1985 Nov 7) 820 (2) 157-64. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.
- AB Chlorpromazine interacted preferentially with membrane proteins rather than membrane lipids in the initial incorporation into human erythrocyte ghosts, as demonstrated by means of the fluorescence quenching and a **maleimide** spin label. In this state the membrane fluidity increased. At higher concentrations of chlorpromazine, the membrane fluidity decreased and a motionally restricted signal from fatty acid spin labels appeared predominantly. However, no such signal appeared in protein-free vesicles. The temperature and pH dependences of the outer hyperfine splitting of this restricted signal were very similar to those of bovine **serum albumin**. On the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of chlorpromazine-treated and -untreated ghosts, it was found that there was no significant difference in membrane proteins between both samples except for the changes of a few bands which were not directly concerned with the occurrence of this restricted signal. These results suggest that the fatty acid spin labels bind preferably to membrane proteins as the lipid domain becomes packed with chlorpromazine.
- L8 ANSWER 20 OF 28 MEDLINE on STN
84291236. PubMed ID: 6470624. Synthesis and evaluation of luminescent

optimised. The three substances used for labelling were diazoluminol, diazoisoluminol and N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide the latter being used as an N-hydroxysuccinamide "active" ester. The ratio of label to IgG was studied for diazoluminol and N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide active ester after it had been discovered that diazoisoluminol was not suitable for coupling to antibodies. The optimal molar ratios label: IgG were for diazoluminol 40:1 and for N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide active ester 60:1. Increasing the substitution rate led to a lessening of the dynamic range, shown by an increase in the ratio between unspecific binding (noise) to maximal binding (signal) in an assay. The synthesis of hapten-protein conjugates for covalent coupling to polystyrene balls was undertaken as this formed part of the preparation for the assays described in Part III. The optimal production of gentamicin-bovine **serum albumin** and thyroxine-transferrin conjugates has been described in detail.

L8 ANSWER 21 OF 28 MEDLINE on STN

83108978. PubMed ID: 6185490. Evidence that inhibitors of anion exchange induce a transmembrane conformational change in band 3. Macara I G; Kuo S; Cantley L C. Journal of biological chemistry, (1983 Feb 10) 258 (3) 1785-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The transport inhibitor, eosin 5-**maleimide**, reacts specifically at an external site on the membrane-bound domain of the anion exchange protein, Band 3, in the human erythrocyte membrane. The fluorescence of eosin-labeled resealed ghosts or intact cells was found to be resistant to quenching by CsCl, whereas the fluorescence of labeled inside-out vesicles was quenched by about 27% at saturating CsCl concentrations. Since both Cs+ and eosin **maleimide** were found to be impermeable to the red cell membrane and the vesicles were sealed, these results indicate that after binding of the eosin **maleimide** at the external transport site of Band 3, the inhibitor becomes exposed to ions on the cytoplasmic surface. The lifetime of the bound eosin **maleimide** was determined to be 3 ns both in the absence and presence of CsCl, suggesting that quenching is by a static rather than a dynamic (collisional) mechanism. Intrinsic tryptophan fluorescence of erythrocyte membranes was also investigated using anion transport inhibitors which do not appreciably absorb light at 335 nm. Eosin **maleimide** caused a 25% quenching and 4,4'-dibenzamidodihydrostilbene-2,2'-disulfonate) caused a 7% quenching of tryptophan fluorescence. Covalent labeling of red cells by either eosin **maleimide** or BIDS (4-benzamido-4'-isothiocyanostilbene-2,2'-disulfonate) caused an increase in the susceptibility of membrane tryptophan fluorescence to quenching by CsCl. The quenching constant was similar

AB An antibody directed toward pepleomycin, a new antitumor antibiotic related structurally to bleomycin, has been produced in rabbits by immunization with a pepleomycin-protein conjugate which was prepared by a novel procedure of coupling pepleomycin to mercaptosuccinylated bovine **serum albumin** using N-(gamma-maleimidobutyryloxy)succinimide as a coupling agent. The antiserum was monospecific to pepleomycin and showed almost no cross-reactivity with a variety of other bleomycin analogs. An enzyme immunoassay for pepleomycin has been developed utilizing this antiserum and beta-D-galactosidase-labeled pepleomycin. The lower limit of detection by this assay, which involves a double antibody technique for the separation of antibody-bound and free antigen, was 50 pg of pepleomycin per tube. Using this assay, drug levels were easily determined in blood and urine of rabbits following administration of pepleomycin in a single dose of 1.2 mg/kg i.v. This assay is also suitable for measuring pepleomycin in the presence of other drugs since the assay is not significantly affected by any other antineoplastic agents tested. Since pepleomycin is now undergoing clinical trial, the enzyme immunoassay of the drug will be a valuable tool in clinical **pharmacological** studies.

L8 ANSWER 24 OF 28 MEDLINE on STN

80226859. PubMed ID: 6248464. Initial kinetics of lysosomal enzyme secretion and superoxide anion generation by human polymorphonuclear leukocytes. Smolen J E; Korchak H M; Weissmann G. Inflammation, (1980 Jun) 4 (2) 145-63. Journal code: 7600105. ISSN: 0360-3997. Pub. country: United States. Language: English.

AB Human polymorphonuclear leukocytes (PMN) exposed to particulate and soluble stimuli secrete lysosomal enzymes. These stimuli cause prompt (less than 10 sec) changes in membrane potential followed 30--45 sec later by superoxide anion (O₂⁻) production. We describe a new technique utilizing flow dialysis apparatus which monitors the first stages of lysosomal enzyme release with a resolution of approximately 6 sec. Secretion of beta-glucuronidase from cytochalasin B-treated PMN could be detected 19+/-5 sec after exposure to the chemotactic peptide N-formylmethionylleucylphenylalanine (FMLP). The "lag" times for release of this enzyme were different for other stimuli: 35+/-8 sec (BSA/anti-BSA immune complex); 48+/-8 sec (serum-treated zymosan, "STZ"); 60+/-25 sec (calcium ionophore A23187). The lag times for lysozyme release were less dependent upon the stimulus presented (28+/-16 sec for FMLP, 28+/-8 sec for BSA/anti-BSA, 32+/-10 sec for STZ, and 38+/-8 seconds for Con A); only A23187 had a long lag period: 74+/-27 sec. Lag periods for the onset of O₂⁻ production (measured by the same mathematical criteria) were comparable to those for beta-glucuronidase release: 21+/-4 sec for FMLP, 43+/-14 sec for BSA/anti-BSA, 6

denaturation of the protein. Chelating reagents such as ethylenediamine tetraacetic acid, 8-hydroxyquinoline inhibited "total", "Cu++ induced" and "thermal" denaturation of the protein. Au+ inhibited "total denaturation", but not "Cu++ induced denaturation". On the other hand, Au+++ denaturated the protein considerably with or without heating, in the absence of Cu++ but dithiothreitol did so only with heating in the same condition. The anti-inflammatory drugs used herein had no effect on the protein denaturation. D-Penicillamine apparently prevents the denaturation of human gamma-globulin by the chelate formation with Cu++ and the binding to free protein SH, initiator for sulphydryldisulfide interchange reaction.

L8 ANSWER 26 OF 28 MEDLINE on STN

78162537. PubMed ID: 347850. [Immobilization of proteins on macroporous glasses involving maleinimide as the anchoring group]. Immobilisierung von Proteinen an makroporösen Glasern unter Beteiligung von Maleinimid als Ankergruppe. Fischer J; Heyer W; Janowski F; Wolf F; Schellenberger A. Acta biologica et medica Germanica, (1977) 36 (7-8) 999-1005. Journal code: 0370276. ISSN: 0001-5318. Pub. country: GERMANY, EAST: German Democratic Republic. Language: German.

AB Macroporous glasses with pore sizes from 400-1000 Å appropriate for protein binding were produced and characterized by a thermal demixing procedure and alkaline after treatment. To achieve a covalent binding capacity relative to proteins, the gamma-aminopropyl derivative was allowed to react with 4-maleinimido benzoylic chloride to give preparations containing, in addition to maleinimide residues, acid chloride structures for the protein binding. A preparation of 400 Å pore size was tested for its protein binding capacity relative to bovine serum albumin and trypsin. Furthermore, the capacity of binding glucoamylase from Endomycopsis bispora in active form was studied.

L8 ANSWER 27 OF 28 MEDLINE on STN

75187762. PubMed ID: 166896. Degradation of insulin by isolated rat liver cells. Le Cam A; Freychet P; Lenoir P. Diabetes, (1975 Jun) 24 (6) 566-73. Journal code: 0372763. ISSN: 0012-1797. Pub. country: United States. Language: English.

AB The degradation of insulin by isolated rat liver cells has been studied. The phenomenon is time- and temperature-dependent. After sixty minutes' exposure to 1.5 times 10⁶ cells/ml, about 50 per cent, 15 per cent, and less than 5 per cent of insulin at 1.5 µM. are degraded at 37 degrees

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L3      39 S L2 AND PY<2000
L4      15041 S L1 AND PHARMACOLOG?
L5      1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6      2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7      47 S L4 AND (MALEIMID?)
L8      28 S L7 AND PY<2000

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>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

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>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATAL. Type FILE USPATAL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATAL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

```

This file contains CAS Registry Numbers for easy and accurate
substance identification.

```

=> s (serum albumin)
      142489 SERUM
      83951 ALBUMIN
L9      59637 (SERUM ALBUMIN)
          (SERUM(W)ALBUMIN)

```

=> d his

(FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005

```

L1      68146 S (SERUM ALBUMIN)
L2      74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3      39 S L2 AND PY<2000
L4      15041 S L1 AND PHARMACOLOG?
L5      1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6      2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7      47 S L4 AND (MALEIMID?)
L8      28 S L7 AND PY<2000

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FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005

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L9      59637 S (SERUM ALBUMIN)

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=> s 19 and (CYS-34 or cysteine-34)

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      47686 CYS
      2089801 34
          87 CYS-34
          (CYS(W)34)
      57397 CYSTEINE
      2089801 34
          20 CYSTEINE-34
          (CYSTEINE(W)34)

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L10 80 L9 AND (CYS-34 OR CYSTEINE-34)

=> s 110 and ay<2000
3004790 AY<2000

L11 13 L10 AND AY<2000

=> d 111,cbib,ab,clm,1-13

L11 ANSWER 1 OF 13 USPATFULL on STN

2003:314573 Oligonucleotides conjugated to protein-binding drugs.

Manoharan, Muthiah, Carlsbad, CA, United States

ISIS Pharmaceuticals, Inc., Carlsbad, CA, United States (U.S. corporation)

US 6656730 B1 20031202

APPLICATION: US 1999-334130 19990615 (9)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Ligand-conjugated oligomeric compounds are described wherein ligands are conjugated to one or more sites on an oligomeric compound including the 2'-, 3'-, 5'-, nucleobase and internucleotide linkage sites. The ligand can be attached via an optional linking group. Ligands are selected for conjugation that bind to one or more cellular, serum or vascular proteins imparting enhanced pharmacokinetic properties to the resulting ligand-conjugated oligomeric compounds. Also provided are methods for increasing the concentration of an oligonucleotide in serum and methods for increasing the capacity of serum for an oligonucleotide. Further, methods for increasing the binding of an oligonucleotide to a portion of the vascular system is described. Also provided are methods for promoting cellular uptake of an oligonucleotide in cells.

CLM What is claimed is:

1. An oligonucleotide covalently attached to an arylpropionic acid that interacts with human **serum albumin**, wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen, ketoprofen, (S)-(+)-pranoprofen, and carprofen.
2. The oligonucleotide of claim 1 wherein said arylpropionic acid binds to said human **serum albumin**.
3. The oligonucleotide of claim 1 wherein said oligonucleotide comprises a plurality of nucleosides connected by covalent internucleoside linkages.
4. The oligonucleotide of claim 3 wherein said linkages are phosphodiester linkages.
5. The oligonucleotide of claim 3 wherein said linkages are phosphorothioate linkages.
6. The oligonucleotide of claim 3 wherein said linkages are non-phosphorus-containing linkages.
7. The oligonucleotide of claim 3 wherein at least one of said nucleosides bears a 2'-substituent group.
8. The oligonucleotide of claim 7 wherein said 2'-substituent group is O-alkylalkoxy.
9. The oligonucleotide of claim 8 wherein said 2'-substituent group is methoxyethoxy.
10. A method of increasing the concentration of an oligonucleotide in human serum comprising the steps of: (a) selecting an arylpropionic acid that is known to bind to human **serum albumin**; (b) covalently-attaching said arylpropionic acid to said oligonucleotide to form a conjugated oligonucleotide; and (c) adding said conjugated oligonucleotide to said human serum, wherein the concentration of said oligonucleotide in human serum is increased; and wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen, ketoprofen, (S)-(+)-pranoprofen, and carprofen.
11. The method claim 10 wherein said arylpropionic acid is ibuprofen.
12. A method of increasing the capacity of human serum for an oligonucleotide comprising the steps of: (a) selecting an arylpropionic acid that is known to bind to human **serum albumin**; (b) covalently attaching said arylpropionic acid to said oligonucleotide to form a conjugated oligonucleotide; and (c) adding said conjugated oligonucleotide to said human serum, wherein the capacity of human serum is increased for said oligonucleotide; and wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen,

ketoprofen, (S)-(+)-pranoprofen, and carprofen.

13. The method of claim 11 wherein said human **serum albumin** has a binding site for said oligonucleotide and a binding site for said arylpropionic acid; wherein said binding site for said oligonucleotide is distinct from said binding site for said arylpropionic acid.

L11 ANSWER 2 OF 13 USPTAFULL on STN

2003:53885 Precerebellin-like protein.

Young, Paul, Gaithersburg, MD, United States
Greene, John M., Gaithersburg, MD, United States
Ferrie, Ann M., Tewksbury, MA, United States
Ruben, Steven M., Olney, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Hu, Jing-Shan, Sunnyvale, CA, United States
Olsen, Henrik S., Gaithersburg, MD, United States
Ebner, Reinhard, Gaithersburg, MD, United States
Brewer, Laurie A., St. Paul, MN, United States
Moore, Paul A., Germantown, MD, United States
Shi, Yanggu, Gaithersburg, MD, United States
Florence, Charles, Rockville, MD, United States
Florence, Kimberly, Rockville, MD, United States
Lafleur, David W., Washington, DC, United States
Ni, Jian, Rockville, MD, United States
Fan, Ping, Gaithersburg, MD, United States
Wei, Ying-Fei, Berkeley, CA, United States
Fischer, Carrie L., Burke, VA, United States
Soppet, Daniel R., Centreville, VA, United States
Li, Yi, Sunnyvale, CA, United States
Zeng, Zhizhen, Gaithersburg, MD, United States
Kyaw, Hla, Frederick, MD, United States
Yu, Guo-Liang, Berkeley, CA, United States
Feng, Ping, Gaithersburg, MD, United States
Dillon, Patrick J., Carlsbad, CA, United States
Endress, Gregory A., Potomac, MD, United States
Carter, Kenneth C., North Potomac, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

US 6525174 B1 20030225

APPLICATION: US 1998-205258 19981204 (9)

PRIORITY: US 1997-70923P 19971218 (60)

US 1998-94657P 19980730 (60)
US 1997-48885P 19970606 (60)
US 1997-49375P 19970606 (60)
US 1997-48881P 19970606 (60)
US 1997-48880P 19970606 (60)
US 1997-48896P 19970606 (60)
US 1997-49020P 19970606 (60)
US 1997-48876P 19970606 (60)
US 1997-48895P 19970606 (60)
US 1997-48884P 19970606 (60)
US 1997-48894P 19970606 (60)
US 1997-48971P 19970606 (60)
US 1997-48964P 19970606 (60)
US 1997-48882P 19970606 (60)
US 1997-48899P 19970606 (60)
US 1997-48893P 19970606 (60)
US 1997-48900P 19970606 (60)
US 1997-48901P 19970606 (60)
US 1997-48892P 19970606 (60)
US 1997-48915P 19970606 (60)
US 1997-49019P 19970606 (60)
US 1997-48970P 19970606 (60)
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US 1997-48916P 19970606 (60)
US 1997-49373P 19970606 (60)
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US 1997-48917P 19970606 (60)
US 1997-48949P 19970606 (60)
US 1997-48974P 19970606 (60)
US 1997-48883P 19970606 (60)
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US 1997-48962P 19970606 (60)
US 1997-48963P 19970606 (60)
US 1997-48877P 19970606 (60)
US 1997-48878P 19970606 (60)

US 1997-57645P 19970905 (60)
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US 1997-57668P 19970905 (60)
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US 1997-57763P 19970905 (60)
US 1997-57650P 19970905 (60)
US 1997-57584P 19970905 (60)
US 1997-57647P 19970905 (60)
US 1997-57661P 19970905 (60)
US 1997-57662P 19970905 (60)
US 1997-57646P 19970905 (60)
US 1997-57654P 19970905 (60)
US 1997-57651P 19970905 (60)
US 1997-57644P 19970905 (60)
US 1997-57765P 19970905 (60)
US 1997-57762P 19970905 (60)
US 1997-57775P 19970905 (60)
US 1997-57648P 19970905 (60)
US 1997-57774P 19970905 (60)
US 1997-57649P 19970905 (60)
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US 1997-57771P 19970905 (60)
US 1997-57761P 19970905 (60)
US 1997-57760P 19970905 (60)
US 1997-57776P 19970905 (60)
US 1997-57778P 19970905 (60)
US 1997-57629P 19970905 (60)
US 1997-57628P 19970905 (60)
US 1997-57777P 19970905 (60)
US 1997-57634P 19970905 (60)
US 1997-70923P 19971218 (60)
US 1998-92921 19980715 (09)
US 1998-94657P 19980730 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CLM What is claimed is:

1. An isolated protein comprising amino acid residues 33 to 205 of SEQ ID NO:463.
2. The isolated protein of claim 1 which comprises amino acid residues 2 to 205 of SEQ ID NO:463.
3. The isolated protein of claim 1 which comprises amino acid residues 1 to 205 of SEQ ID NO:463.
4. The protein of claim 1 which comprises a heterologous polypeptide sequence.
5. A composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.
6. An isolated protein produced by the method comprising: (a) expressing the protein of claim 1 by a cell; and (b) recovering said protein.
7. An isolated protein comprising a polypeptide sequence which is at least 95% identical to amino acid residues 33 to 205 of SEQ ID NO:463.
8. The isolated protein of claim 7 wherein said polypeptide sequence is at least 95% identical to amino acid residues 1 to 205 of SEQ ID NO:463.
9. The protein of claim 7 which comprises a heterologous polypeptide sequence.
10. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.

11. An isolated protein produced by the method comprising: (a) expressing the protein of claim 7 by a cell; and (b) recovering said protein.
12. An isolated protein comprising at least 50 contiguous amino acid residues of amino acid residues 33 to 205 of SEQ ID NO:463.
13. The protein of claim 12 which comprises a heterologous polypeptide sequence.
14. A composition comprising the protein of claim 12 and a pharmaceutically acceptable carrier.
15. An isolated protein produced by the method comprising: (a) expressing the protein of claim 12 by a cell; and (b) recovering said protein.
16. An isolated protein comprising at least 50 contiguous amino acid residues of amino acid residues 1 to 205 of SEQ ID NO:463.
17. The protein of claim 16 which comprises a heterologous polypeptide sequence.
18. A composition comprising the protein of claim 16 and a pharmaceutically acceptable carrier.
19. An isolated protein produced by the method comprising: (a) expressing the protein of claim 16 by a cell; and (b) recovering said protein.

L11 ANSWER 3 OF 13 USPTAFULL on STN
2002:175279 186 human secreted proteins.

Ruben, Steven M., Olney, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Fischer, Carrie L., Burke, VA, United States
Soppet, Daniel P., Centreville, VA, United States
Carter, Kenneth C., North Potomac, MD, United States
Bednarik, Daniel R., Columbia, MD, United States
Endress, Gregory A., Potomac, MD, United States
Yu, Guo-Liang, Berkeley, CA, United States
Ni, Jian, Rockville, MD, United States
Feng, Ping, Gaithersburg, MD, United States
Young, Paul E., Gaithersburg, MD, United States
Greene, John M., Gaithersburg, MD, United States
Ferrie, Ann M., Tewksbury, MA, United States
Duan, Roxanne, Bethesda, MD, United States
Hu, Jing-Shan, Sunnyvale, CA, United States
Florence, Kimberly A., Rockville, MD, United States
Olsen, Henrik S., Gaithersburg, MD, United States
Ebner, Reinhard, Gaithersburg, MD, United States
Brewer, Laurie A., St. Paul, MN, United States
Moore, Paul A., Germantown, MD, United States
Shi, Yanggu, Gaithersburg, MD, United States
Lafleur, David W., Washington, DC, United States
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Zeng, Zhizhen, Lansdale, PA, United States
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US 6420526 B1 20020716

APPLICATION: US 1998-149476 19980908 (9)

PRIORITY: US 1997-40162P 19970307 (60)

US 1997-40333P 19970307 (60)
US 1997-38621P 19970307 (60)
US 1997-40626P 19970307 (60)
US 1997-40334P 19970307 (60)
US 1997-40336P 19970307 (60)
US 1997-40163P 19970307 (60)
US 1997-47600P 19970523 (60)
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US 1997-49610P 19970613 (60)
US 1997-61060P 19971002 (60)
US 1997-51926P 19970708 (60)

US 1997-52874P 19970716 (60)

US 1997-58785P 19970912 (60)

US 1997-55724P 19970818 (60)

US 1997-40161P 19970307 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CLM What is claimed is:

1. An isolated protein comprising amino acid residues 27 to 234 of SEQ ID NO:478.
2. The isolated protein of claim 1 which comprises amino acid residues 2 to 234 of SEQ ID NO:478.
3. The isolated protein of claim 1 which comprises amino acid residues 1 to 234 of SEQ ID NO:478.
4. The protein of claim 1 which comprises a heterologous polypeptide sequence.
5. A composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.
6. An isolated protein produced by the method comprising: (a) expressing the protein of claim 1 by a cell; and (b) recovering said protein.
7. An isolated protein comprising the amino acid sequence of the secreted portion of the polypeptide encoded by the HNF1P24 cDNA contained in ATCC Deposit No. 97903.
8. The isolated protein of claim 7 which comprises the amino acid sequence of the complete polypeptide encoded by the HNF1P24 cDNA contained in ATCC Deposit No. 97903, excepting the N-terminal methionine.
9. The isolated protein of claim 7 which comprises the amino acid sequence of the complete polypeptide encoded by the HNF1P24 cDNA contained in ATCC Deposit No. 97903.
10. The protein of claim 7 which comprises a heterologous polypeptide sequence.
11. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.
12. An isolated protein produced by the method comprising: (a) expressing the protein of claim 7 by a cell; and (b) recovering said protein.
13. An isolated protein comprising a polypeptide sequence which is at least 90% identical to amino acid residues 27 to 234 of SEQ ID NO:478.
14. The isolated protein of claim 13 wherein said polypeptide sequence is at least 90% identical to amino acid residues 1 to 234 of SEQ ID NO:478.
15. The isolated protein of claim 13 wherein said polypeptide sequence is at least 95% identical to amino acid residues 27 to 234 of SEQ ID NO:478.
16. The isolated protein of claim 13 wherein said polypeptide sequence is at least 95% identical to amino acid residues 1 to 234 of SEQ ID NO:478.
17. The protein of claim 13 which comprises a heterologous polypeptide sequence.
18. A composition comprising the protein of claim 13 and a pharmaceutically acceptable carrier.
19. An isolated protein produced by the method comprising: (a) expressing the protein of claim 13 by a cell; and (b) recovering said protein.

20. An isolated protein comprising a polypeptide sequence which is at least 90% identical to the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
21. The isolated protein of claim 20 wherein said polypeptide sequence is at least 95% identical to the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
22. The protein of claim 20 which comprises a heterologous polypeptide sequence.
23. A composition comprising the protein of claim 20 and a pharmaceutically acceptable carrier.
24. An isolated protein produced by the method comprising: (a) expressing the protein of claim 20 by a cell; and (b) recovering said protein.
25. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 27 to 234 of SEQ ID NO:478.
26. The isolated protein of claim 25 which comprises at least 50 contiguous amino acid residues of amino acid residues 27 to 234 of SEQ ID NO:478.
27. The protein of claim 25 which comprises a heterologous polypeptide sequence.
28. A composition comprising the protein of claim 25 and a pharmaceutically acceptable carrier.
29. An isolated protein produced by the method comprising: (a) expressing the protein of claim 25 by a cell; and (b) recovering said protein.
30. An isolated protein comprising at least 30 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
31. The isolated protein of claim 30 which comprises at least 50 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
32. The protein of claim 30 which comprises a heterologous polypeptide sequence.
33. A composition comprising the protein of claim 30 and pharmaceutically acceptable carrier.
34. An isolated protein produced by the method comprising: (a) expressing the protein of claim 30 by a cell; and (b) recovering said protein.
35. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 1 to 234 of SEQ ID NO:478.
36. The isolated protein of claim 35 which comprises at least 50 contiguous amino acid residues of amino acid residues 1 to 234 of SEQ ID NO:478.
37. The protein of claim 35 which comprises a heterologous polypeptide sequence.
38. A composition comprising the protein of claim 35 and a pharmaceutically acceptable carrier.
39. An isolated protein produced by the method comprising: (a) expressing the protein of claim 35 by a cell; and (b) recovering said protein.
40. An isolated protein comprising at least 30 contiguous amino acid residues of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
41. The isolated protein of claim 40 which comprises at least 50 contiguous amino acid residues of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

42. The protein of claim 40 which comprises a heterologous polypeptide sequence.
43. A composition comprising the protein of claim 40 and pharmaceutically acceptable carrier.
44. An isolated protein produced by the method comprising: (a) expressing the protein of claim 40 by a cell; and (b) recovering said protein.
45. An isolated protein comprising a polypeptide sequence which is at least 90% identical to the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
46. The isolated protein of claim 45, wherein said polypeptide sequence is at least 95% identical to the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.
47. The protein of claim 45 which comprises a heterologous polypeptide sequence.
48. The protein of claim 47, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
49. A composition comprising the protein of claim 45 and a pharmaceutically acceptable carrier.
50. An isolated protein produced by a method comprising: (a) expressing the protein of claim 45 by a cell; and (b) recovering said protein.
51. The protein of claim 4, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
52. The protein of claim 10, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
53. The protein of claim 17, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
54. The protein of claim 22, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
55. The protein of claim 27, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
56. The protein of claim 32, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
57. The protein of claim 37, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.
58. The protein of claim 42, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

L11 ANSWER 4 OF 13 USPTAFULL on STN

2002:81240 Polynucleotides encoding chemokine α -6.

Wei, Ying-Fei, San Mateo, CA, United States

Ruben, Steven M., Olney, MD, United States

Rosen, Craig A., Laytonsville, MD, United States

Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

US 6372456 B1 20020416

APPLICATION: US 1998-177304 19981023 (9)

PRIORITY: US 1997-63387P 19971024 (60)

US 1998-79245P 19980325 (60)

DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a novel CK(α -6 protein which is a member of the alpha chemokine family. In particular, isolated nucleic acid molecules are provided encoding the human CK α -6 protein. CK α -6 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of CK α -6 activity. Also provided are diagnostic methods for detecting CNS and immune system-related disorders and therapeutic methods for treating CNS and immune system-related disorders.

What is claimed is:

1. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +1 to +84 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +2 to +84 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +17 to +84 of SEQ ID NO:2; (d) a nucleic acid sequence encoding amino acid residues +18 to +84 of SEQ ID NO:2; (e) a nucleic acid sequence encoding amino acid residues +19 to +84 of SEQ ID NO:2; (f) a nucleic acid sequence encoding amino acid residues +20 to +84 of SEQ ID NO:2; (g) a nucleic acid sequence encoding amino acid residues +21 to +84 of SEQ ID NO:2; and (h) a nucleic acid sequence encoding amino acid residues +22 to +84 of SEQ ID NO:2.
2. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (a).
3. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (b).
4. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (c).
5. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (d).
6. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (e).
7. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (f).
8. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (g).
9. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (h).
10. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 1.
11. The isolated polynucleotide of claim 1 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.
12. The isolated polynucleotide of claim 11 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.
13. The isolated polynucleotide of claim 12 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.
14. A recombinant vector comprising the isolated polynucleotide of claim 1.
15. The recombinant vector of claim 14 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
16. A recombinant host cell comprising the isolated polynucleotide of claim 1.
17. The recombinant host cell of claim 16 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
18. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 1; and (b) recovering the protein from the cell culture.
19. A composition comprising the isolated polynucleotide of claim 1 and a pharmaceutically acceptable carrier.
20. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide encoded by the cDNA in ATCC Deposit No. 209643; (b) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 209643; and (c) a nucleic acid sequence encoding the amino

acid sequence of the mature polypeptide encoded by the cDNA in ATCC Deposit No. 209643.

21. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (a).

22. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (b).

23. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (c).

24. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 20.

25. The isolated polynucleotide of claim 20 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

26. The isolated polynucleotide of claim 25 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

27. The isolated polynucleotide of claim 26 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

28. A recombinant vector comprising the isolated polynucleotide of claim 20.

29. The recombinant vector of claim 28 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

30. A recombinant host cell comprising the isolated polynucleotide of claim 20.

31. The recombinant host cell of claim 30 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

32. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 20; and (b) recovering the protein from the cell culture.

33. A composition comprising the isolated polynucleotide of claim 20 and a pharmaceutically acceptable carrier.

34. An isolated polynucleotide comprising a first nucleic acid sequence 90% or more identical to a second nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +1 to +84 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +2 to +84 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +17 to +84 of SEQ ID NO:2; (d) a nucleic acid sequence encoding amino acid residues +18 to +84 of SEQ ID NO:2; (e) a nucleic acid sequence encoding amino acid residues +19 to +84 of SEQ ID NO:

40. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (f).
41. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (g).
42. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (h).
43. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (a).
44. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (b).
45. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (c).
46. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (d).
47. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (e).
48. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (f).
49. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (g).
50. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (h).
51. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 34.
52. The isolated polynucleotide of claim 34 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.
53. The isolated polynucleotide of claim 52 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.
54. The isolated polynucleotide of claim 53 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.
55. A recombinant vector comprising the isolated polynucleotide of claim 34.
56. The recombinant vector of claim 55 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
57. A recombinant host cell comprising the isolated polynucleotide of claim 34.
58. The recombinant host cell of claim 57 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
59. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 34; and (b) recovering the protein from the cell culture.
60. A composition comprising the isolated polynucleotide of claim 34 and a pharmaceutically acceptable carrier.
61. An isolated polynucleotide comprising a first nucleic acid sequence 90% or more identical to a second nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide encoded by the cDNA in ATCC Deposit No. 209643; (b) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 209643; and (c) a nucleic acid sequence encoding the amino acid sequence of the mature polypeptide encoded by the cDNA in ATCC Deposit No. 209643; wherein percent identity is calculated using FASTDB with the parameters set such that percentage of identity is calculated over the full length of the reference nucleic acid sequence and that gaps in homology of up

to 5% of the total number of nucleic acids in the reference nucleic acid sequence are allowed.

62. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (a).

63. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (b).

64. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (c).

65. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (a).

66. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (b).

67. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (c).

68. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 61.

69. The isolated polynucleotide of claim 61 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

70. The isolated polynucleotide of claim 69 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

71. The isolated polynucleotide of claim 70 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

72. A recombinant vector comprising the isolated polynucleotide of claim 61.

73. The recombinant vector of claim 72 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

74. A recombinant host cell comprising the isolated polynucleotide of claim 61.

75. The recombinant host cell of claim 74 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

76. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 61; and (b) recovering the protein from the cell culture.

77. A

84. A recombinant host cell comprising the isolated polynucleotide of claim 78.
85. The recombinant host cell of claim 84 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
86. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 78; and (b) recovering the protein from the cell culture.
87. A composition comprising the isolated polynucleotide of claim 78 and a pharmaceutically acceptable carrier.
88. An isolated polynucleotide comprising a nucleic acid sequence which hybridizes to the cDNA in ATCC Deposit No. 209643 wherein said hybridization occurs under conditions comprising hybridization in a buffer consisting essentially of 50% formamide, 5×SSC, 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 ug/ml denatured, sheared salmon sperm DNA at 42° C. and wash in a solution consisting of 0.1×SSC at 65°.
89. The isolated polynucleotide of claim 88 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.
90. The isolated polynucleotide of claim 89 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.
91. The isolated polynucleotide of claim 90 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.
92. A recombinant vector comprising the isolated polynucleotide of claim 88.
93. The recombinant vector of claim 92 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
94. A recombinant host cell comprising the isolated polynucleotide of claim 88.
95. The recombinant host cell of claim 94 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.
96. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 88; and (b) recovering the protein from the cell culture.
97. A composition comprising the isolated polynucleotide of claim 88 and a pharmaceutically acceptable carrier.
98. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +26 to +34 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +36 to +45 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +58 to +66 of SEQ ID NO:2; and (d) a nucleic acid sequence encoding amino acid residues +77 to +84 of SEQ ID NO:2.
99. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (a).
100. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (b).
101. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (c).
102. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (d).
103. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 98.
104. The isolated polynucleotide of claim 98 wherein said nucleic acid

sequence further comprises a heterologous nucleic acid sequence.

105. The isolated polynucleotide of claim 104 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

106. The isolated polynucleotide of claim 105 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

107. A recombinant vector comprising the isolated polynucleotide of claim 98.

108. The recombinant vector of claim 107 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

109. A recombinant host cell comprising the isolated polynucleotide of claim 98.

110. The recombinant host cell of claim 109 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

111. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 98; and (b) recovering the protein from the cell culture.

112. A composition comprising the isolated polynucleotide of claim 98 and a pharmaceutically acceptable carrier.

113. An isolated polynucleotide comprising a nucleic acid sequence which encodes at least 30 contiguous amino acid residues of SEQ ID NO:2.

114. The isolated polynucleotide of claim 113 which comprises a nucleic acid sequence which encodes at least 50 contiguous amino acid residues of SEQ ID NO:2.

115. The isolated polynucleotide of claim 113 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

116. The isolated polynucleotide of claim 115 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

117. The isolated polynucleotide of claim 116 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

118. A recombinant vector comprising the isolated polynucleotide of claim 113.

119. The recombinant vector of claim 118 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

120. A recombinant host cell comprising the isolated polynucleotide of claim 113.

127. The isolated polynucleotide of claim 126 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

128. The isolated polynucleotide of claim 127 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

129. A recombinant vector comprising the isolated polynucleotide of claim 124.

130. The recombinant vector of claim 129 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

131. A recombinant host cell comprising the isolated polynucleotide of claim 124.

132. The recombinant host cell of claim 131 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

133. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 124; and (b) recovering the protein from the cell culture.

134. A composition comprising the isolated polynucleotide of claim 124 and a pharmaceutically acceptable carrier.

135. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues n to 84 of SEQ ID NO:2, where n is an integer in the range of 17 to 22; (b) a nucleic acid sequence encoding amino acid residues 17 to m of SEQ ID NO:2, where m is an integer in the range of 68 to 84; and (c) a nucleic acid sequence encoding amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 17 to 22 and m is an integer in the range of 68 to 84; wherein said nucleic acid sequence is not Genbank Accession No. AA410918.

136. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (a).

137. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (b).

138. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (c).

139. The isolated polynucleotide of claim 135 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

140. The isolated polynucleotide of claim 139 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

selected from the group consisting of: (a) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment inhibits endothelial cell function; (b) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment inhibits inflammation; (c) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment is angiostatic; and (d) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment binds an antibody specific for the polypeptide encoded by SEQ ID NO:2.

149. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (a).

150. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (b).

151. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (c).

152. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (d).

153. The isolated polynucleotide of claim 148 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

154. The isolated polynucleotide of claim 153 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

155. The isolated polynucleotide of claim 154 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

156. A recombinant vector comprising the isolated polynucleotide of claim 148.

157. The recombinant vector of claim 156 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

158. A recombinant host cell comprising the isolated polynucleotide of claim 148.

159. The recombinant host cell of claim 158 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

160. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 148; and (b) recovering the protein from the cell culture.

161. A composition comprising the isolated polynucleotide of claim 148 and a pharmaceutically acceptable carrier.

L11 ANSWER 5 OF 13 USPTAFULL on STN

3. A pharmaceutical preparation according to claim 2, wherein said thiol-group-containing proteins comprises at least one selected from the group consisting of albumin, orosomucoid, tissue-plasminogen activator, fibrinogen, Lys-plasminogen, and hemoglobin.
4. A pharmaceutical preparation according to claim 1, wherein at least 60% of said thiol groups of said thiol-group-containing proteins are nitrosated.
5. A pharmaceutical preparation according to claim 1, wherein 65% to 95% of said thiol groups of said thiol-group-containing proteins are nitrosated.
6. A pharmaceutical preparation according to claim 1, wherein 70% to 90% of said thiol groups of said thiol-group-containing proteins are nitrosated.
7. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins are human S-nitroso-albumin and hemoglobin.
8. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 30%.
9. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 20%.
10. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 10%.
11. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins have been purified.
12. A pharmaceutical preparation according to claim 11, wherein said thiol-group-containing proteins are at least 80% of the total protein content of the preparation.
13. A pharmaceutical preparation according to claim 11, wherein said thiol-group-containing proteins are at least 90% of the total protein content of the preparation.
14. A pharmaceutical preparation according to claim 4, wherein said pharmaceutical preparation has a stability corresponding to an in vivo half-life of at least 20 minutes.
15. A pharmaceutical preparation according to claim 4, wherein said pharmaceutical preparation has a stability corresponding to an in vivo half-life of 40 minutes to 2 hours.
16. A pharmaceutical preparation according to claim 1, wherein said pharmaceutical preparation is in frozen form.
17. A pharmaceutical preparation according to claim 1, wherein said pharmaceutical preparation is in lyophilized form.
18. A method for preparing a stable, virus-safe pharmaceutical preparation, said method comprising heat treating a composition comprising thiol-group-containing proteins, and processing said thiol-group-containing proteins so as to provide processed proteins with at least 40% of said thiol groups in a form capable of being nitrosated.
19. A method according to claim 18, further comprising nitrosating said thiol groups of said processed proteins with a nitrosating agent.
20. A method according to claim 18, wherein said processing is treating said thiol-group-containing proteins with a reducing agent.
21. A method according to claim 20, wherein said reducing agent is a monothiol-group-containing compound.
22. A method according to claim 20, wherein said reducing agent is β -mercapto-ethanol.
23. A method according to claim 18, wherein said processing is performed at a temperature below 20° C.

24. A method according to claim 18, wherein said processing is performed at a temperature between 2° C. to 100° C.

25. A method according to claim 18, wherein said processing is performed for 1 to 100 hours.

26. A method according to claim 18, wherein said processing is performed for 12 to 48 hours.

27. A method according to claim 18, wherein said composition is selected from the group consisting of plasma, serum, a plasma fraction and a purified protein preparation.

28. A method according to claim 18, further comprising at least one step selected from the group consisting of precipitation, gel filtration, ultrafiltration and chromatographic purification.

29. A method according to claim 18, further comprising at least one purification step after said processing.

30. A method according to claim 19, further comprising at least one purification step after said nitrosating of said thiol groups.

31. A method according to claim 29, wherein said purification step is chromatographic purification.

32. A method according to claim 19, wherein said nitrosating of said thiol groups is carried out under aerobic and acidic conditions with an agent selected from the group consisting of HNO₂, HNO, NOCl, NO⁺, RNO₂, N₂ O₃, N₂ O₄, NO₂ -- radical and NO-- radical.

33. A method according to claim 19, wherein said nitrosating is performed by adding a nitrosating agent in the range of 0.5 to 6 parts agent per part thiol-group-containing protein based upon thiol group content.

34. A method according to claim 19, wherein said nitrosating is performed by adding a nitrosating agent in the range of 1 to 2 parts agent per part thiol-group-containing protein based upon thiol group content.

L11 ANSWER 6 OF 13 USPTAFULL on STN

2000:4924 Peptide variants of protein A.

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consisting of Lys and Arg; and X₂ is selected from the group consisting of OR₁ and NR₁ R₂ where R₁ and R₂ are independently selected from the group consisting of H, C₁-C₆ alkyl, C₆-C₁₂ aryl and C₆-C₁₂ aryl-C₁-C₆ alkyl.

2. The compound of claim 1 selected from the group consisting of Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Lys-Glu-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His-

(SEQ ID NO:5)

Asp-Pro-As

n-Leu-Asn-Gl

u-

Glu-Gln-Arg-

As

n-Ala-Lys-Il

e-

Gln-Ser-Ile-

Ly

s-Asp-Asp-X.

su

b.2 ;

Z-Ala-Val-

Arg-Asn-Gly-

Ph

e-Asn-Lys-Gl

u-

Gln-Gln-Asn-

Ar

g-Phe-Tyr-Gl

u-

Ala-Leu-His-

(SEQ ID

NO:6)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-

A

sp-Asp-X.sub

.2

;

- Z-Ala-Val-Ala-Gln-Ser-Phe-Asn-Lys-Glu-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-

Ala

-Leu-His-

(SEQ ID

NO:7)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-

A

sp-Asp-X.sub

.2

;

- Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Met-Gln-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-

Ala

-Leu-His-

(SEQ ID

NO:8)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-

A

sp-Asp-X.sub

.2

;

- Z-Ala-Val-Asp-Asn-Lys-Gly-Trp-Met-Arg-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-

Ala

-Leu-His-

(SEQ ID

NO:9)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-

A

sp-Asp-X.sub

.2

;

- Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Lys-Glu-Gln-Gln-Arg-Arg-Phe-Tyr-Glu-

Ala

-Leu-His-

(SEQ ID

NO:10)

-

Asp-Pro-As

n-Leu-Asn-Gl

u-	Glu-Gln-Arg-
As	n-Ala-Lys-I1
e-	Gln-Ser-Ile-
Ly	s-Asp-Asp-X.
su	b.2 ;
	-
Z-Ala-Val-	Asp-Asn-Lys-
Ph	e-Asn-Lys-Gl
u-	Gln-Gln-Arg-
Al	a-Phe-Tyr-Gl
u-	Ala-Leu-His-
	(SEQ ID
	NO:11)
	-
Asp-Pro-As	n-Leu-Asn-Gl
u-	Glu-Gln-Arg-
As	n-Ala-Lys-I1
e-	Gln-Ser-Ile-
Ly	s-Asp-Asp-X.
su	b.2 ;
	-
Z-Ala-Val-	Asp-Asn-Lys-
Ph	e-Asn-Lys-Gl
u-	Gln-Gln-Asn-
Ar	g-Phe-Tyr-Gl
u-	Ala-Leu-His-
	(SEQ ID
	NO:12)
	-
Asp-Pro-As	n-Leu-Asn-Gl
u-	Glu-Gln-Arg-
As	n-Ala-Lys-I1
e-	Lys-Ser-Ile-
Ar	g-Asp-Asp-X.
su	b.2 ;
	-
Z-Ala-Val-	Ala-Asn-Gly-
Ph	e-Asn-Met-Gl
u-	Gln-Gln-Arg-
Ar	g-Phe-Tyr-Gl
u-	Ala-Leu-His-
	(SEQ ID
	NO:13)
	-
Asp-Pro-As	n-Leu-Asn-Gl
u-	

As	Glu-Gln-Arg-
e-	n-Ala-Lys-Il
Ar	Lys-Ser-Ile-
su	g-Asp-Asp-X.
	b.2 ;
	-
Z-Ala-Val-	Ala-Gln-Ser-
Ph	e-Asn-Met-Gl
u-	Gln-Gln-Arg-
Ar	g-Phe-Tyr-Gl
u-	Ala-Leu-His-
	(SEQ ID
	NO:14)
	-
Asp-Pro-As	n-Leu-Asn-Gl
u-	Glu-Gln-Arg-
As	n-Ala-Lys-Il
e-	Lys-Ser-Ile-
Ar	g-Asp-Asp-X.
su	b.2 ;
	- X ₁
	-Phe-Asn-Met
-G	ln-Gln-Gln-A
rg	-Arg-Phe-Tyr
-G	lu-Ala-Leu-H
is	-Asp-Pro-Asn
-L	eu-Asn-
(SEQ	ID NO:15)
	-
Glu-Glu-Gl	n-Arg-Asn-Al
a-	Lys-Ile-Lys-
Se	r-Ile-Arg-As
p-	Asp-X ₂
;	
- Z-Ala-Val-Ala-Gln-Ser-Phe-Asn-Met-Glu-Gln-Gln-Ala-Arg-Phe-Tyr-Glu-	
Ala	-Leu-His-
	(SEQ ID
	NO:24)
	-
Asp-Pro-As	n-Leu-Asn-Gl
u-	Glu-Gln-Arg-
As	n-Ala-Lys-Il
e-	Lys-Ser-Ile-
Ar	g-Asp-Asp-X.
su	b.2 ; and
	- X ₁
	-Phe-Asn-Met

-G	ln-Gln-Gln-A
la	-Arg-Phe-Tyr
-G	lu-Ala-Leu-H
is	-Asp-Pro-Asn
-L	eu-Asn- (SEQ ID NO:25)
	-
Glu-Glu-Gl	n-Arg-Asn-Al
a-	Lys-Ile-Lys-
Se	r-Ile-Arg-As
p-	Asp-X ₂

; where Z is selected from the group consisting of H and C₁ -C₆ alkanoyl; X₁ is selected from the group consisting of H and C₁ -C₆ alkanoyl; and X₂ is selected from the group consisting of OR₁ and NR₁ R₂ where R₁ and R₂ are independently selected from the group consisting of H, C₁ -C₆ alkyl, C₆ -C₁₂ aryl and C₆ -C₁₂ aryl-C₁ -C₆ alkyl.

3. The compound of claim 1 that is covalently linked to a macromolecule.
4. The compound of claim 3 wherein the macromolecule is a solid support.
5. The compound of claim 1 that is fused to a selected polypeptide to form a fusion protein.
6. The fusion protein of claim 5 wherein the compound of Formula (I) is specifically cleavable from the selected polypeptide.

7. A compound represented by Formula (II):

-AA ₆ -AA ₇ -AA ₈ -AA ₉ -Cys-Gln-AA.s	X ₁
ub.12 -AA ₁₃ -Phe-Tyr-Glu-Ala-Leu-His-Asp-Pro-Asn	

(II)

.vertline.	.vertline.
	S-----
--	-----S
	.vertline.
	.vertli
ne	.
	.vertline.
	(SEQ ID NO: 3)
	X ₂
	-Cys-Asp-Asp-AA.
su	b.36
-Ile-Ser-AA.s	ub.33
-Ile-Lys-Ala	-Asn-Arg-Gln-Glu
-G	lu-Asn-Leu

where X₁ is selected from the group consisting of H, C₁ -C₆ alkanoyl, and Z-Ala-Val-AA₃ -AA₄ AA₅ (SEQ ID NO:2); where Z is selected from the group consisting of H and C₁ -C₆ alkanoyl; AA₃ is selected from the group consisting of Asp, Arg, and Ala; AA₄ is selected from the group consisting of Asn and Gln; and AA₅ is selected from the group consisting of Lys, Gly, and Ser; AA₆ is selected from the group consisting of Phe and Gly; AA₇ is selected from the group consisting of Asn and Trp; AA₈ is selected from the group consisting of Lys and Met; AA₉ is selected from the group consisting of Glu, Gln, and Arg; AA₁₂ is selected from the group consisting of Asn, Ala, and Arg; AA₁₃ is selected from the group consisting of Ala and Arg; AA₃₃ is selected from the group consisting of Gln and Lys; AA₃₆ is selected from the

group consisting of Lys and Arg; and X₂ is selected from the group consisting of OR₁ and NR₁ R₂ where R₁ and R₂ are independently selected from the group consisting of H, C₁-C₆ alkyl, C₆-C₁₂ aryl and C₆-C₁₂ aryl-C₁-C₆ alkyl.

8. The compound of claim 7 wherein X₁ is Z-Ala-Val-AA₃-AA₄-AA₅ (SEQ ID NO:2); AA₃ is Arg; and AA₅ is Gly.

9. The compound of claim 8 wherein AA₄ is Asn; AA₆ is Phe; AA₇ is Asn; AA₈ is Lys; AA₉ is Glu; AA₁₂ is Asn; AA₁₃ is Arg; AA₃₃ is Gln; and AA₃₆ is Lys.

10. The compound of claim 7 wherein X₁ is Z-Ala-Val-AA₃-AA₄-AA₅ (SEQ ID NO:2); AA₃ is Ala; AA₄ is Gln; and AA₅ is Ser.

11. The compound of claim 10 wherein AA₆ is Phe; AA₇ is Asn; AA₈ is Lys; AA₉ is Glu; AA₁₂ is Asn; AA₁₃ is Arg; AA₃₃ is Gln; and AA₃₆ is Lys.

12. The compound of claim 7 wherein AA₈ is Met and AA₉ is Gln.

13. The compound of claim 12 wherein X₁ is Z-Ala-Val-AA₃-AA₄-AA₅ (SEQ ID NO:2); AA₃ is Asp; AA₄ is Asn; AA₅ is Lys; AA₆ is Phe; AA₇ is Asn; AA₁₂ is Asn; AA₁₃ is Arg; AA₃₃ is Gln; and AA₃₆ is Lys.

14. The compound of claim 7 wherein AA₆ is Gly; AA₇ is Trp; AA₈ is Met; and AA₉ is Arg.

27. The compound of claim 26 wherein AA₆ is Phe; AA₇ is Asn;
and AA₁ 3 is Arg.

L11 ANSWER 7 OF 13 USPATFULL on STN

1999:137461 Platelet substitutes and conjugation methods suitable for their
preparation.

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US 5977313 19991102

APPLICATION: US 1997-953514 19971017 (8)

PRIORITY: GB 1996-21886 19961010

GB 1997-2652 19970210

DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Platelet substitutes, comprising fibrinogen, or analogous products
useful in therapy, which further comprise an insoluble carrier to which
is bound an essentially non-degraded active protein including the
sequence Arg-Gly-Asp. Such conjugates can be made by a conjugation
process comprising 0.01 to 2.5% by weight active fibrinogen, and no more
than 50% inactive fibrinogen.

CLM What is claimed is:

1. A pharmaceutically-acceptable product comprising an insoluble carrier
to which is bound an essentially non-degraded active protein selected
from fibrinogen and fragments thereof having platelet aggregation
activity.

2. A product according to claim 1, wherein the binding is by

3. The composition of claim 1 wherein said first and said second oligonucleotides comprise an antisense/sense pair of oligonucleotides.
4. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 10 to 40 nucleotides.
5. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 18 to 30 nucleotides.
6. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 21 to 24 nucleotides.
7. The composition of claim 1 wherein said first oligomeric compound comprises an antisense oligonucleotide.
8. The composition of claim 7 wherein said second oligomeric compound comprises a sense oligonucleotide.
9. The composition of claim 7 wherein said second oligomeric compound comprises an oligonucleotide having a plurality of ribose nucleotide units.
10. The composition of claim 1 wherein said first oligomeric compound comprises said at least one conjugate moiety.
11. The composition of claim 1 wherein said second oligomeric compound comprises said at least one conjugate moiety.
12. The composition of claim 1 wherein said first and second oligomeric compounds each comprises at least one conjugate moiety.
13. The composition of claim 1 wherein said second oligomeric compound comprises at least one conjugate moiety and said first oligomeric compound comprises no conjugate moiety.
14. The composition of claim 13 wherein said second oligomeric compound comprises a sense oligonucleotide.
15. The composition of claim 1 wherein said at least one conjugate moiety is attached to an internal residue of said first or second oligomeric compounds.
16. The composition of claim 1 wherein said at least one conjugate moiety is attached to a terminal residue of said first or second oligomeric compounds.
17. The composition of claim 16 wherein said terminal residue is at the 5' end of said first or second oligomeric compounds.
18. The composition of claim 1 wherein said conjugate moiety is attached to a heterocyclic base moiety of said first or second oligomeric compounds.
19. The composition of claim 1 wherein said at least one conjugate moiety is attached to a monomeric subunit of said first or second oligomeric compounds.
20. The composition of claim 1 wherein said at least one conjugate moiety is attached to a monomeric subunit linkage of said first or second oligomeric compounds.
21. The composition of claim 1 wherein said at least one conjugate moiety is attached to said first or second oligomeric compounds through a linker.
22. The composition of claim 1 wherein said at least one conjugate moiety is a lipophilic moiety, vitamin, polymer, peptide, protein, nucleic acid, small molecule, oligosaccharide, carbohydrate cluster, intercalator, minor groove binder, cleaving agent, or cross-linking agent.
23. The composition of claim 1 wherein said at least one conjugate moiety is a steroid.
24. The composition of claim 1 wherein said at least one conjugate moiety is cholesterol or a cholesterol derivative.
25. The composition of claim 1 wherein said at least one conjugate

moiety binds to low-density lipoprotein.

26. The composition of claim 1 wherein said at least one conjugate moiety is folate or folate derivative.

27. The composition of claim 1 wherein said at least one conjugate moiety is a water-soluble polymer.

28. The composition of claim 1 wherein said at least one conjugate moiety comprises polyethylene glycol or copolymer thereof.

29. The composition of claim 1 wherein said at least one conjugate moiety comprises a fusogenic peptide or delivery peptide.

30. The composition of claim 1 wherein said at least one conjugate moiety comprises a nuclear export signal.

31. The composition of claim 1 wherein said at least one conjugate moiety comprises a nucleic acid.

32. The composition of claim 1 wherein said at least one conjugate moiety comprises a drug.

33. The composition of claim 1 wherein said at least one conjugate moiety binds to human **serum albumin**.

34. The composition of claim 1 wherein said at least one conjugate moiety comprises a reporter group.

35. The composition of claim 1 wherein said at least one conjugate moiety localizes said first oligomeric compound, said second oligomeric compound, or both to the cytoplasm of a cell.

36. The composition of claim 1 wherein said at least one conjugate moiety enhances the pharmacokinetic or pharmacodynamic properties of said composition.

37. The composition of claim 1 wherein said composition has improved cellular uptake properties compared with the same composition having no conjugate moiety.

38. A composition comprising, a first oligomeric compound capable of hybridizing to a target nucleic acid, optionally a second oligomeric compound hybridizable to said first oligomeric compound; at least one protein, said protein comprising at least a portion of a RNA-induced silencing complex (RISC), wherein said composition comprises at least one oligomeric compound comprising at least one conjugate moiety.

39. The composition of claim 38 wherein said first oligomeric compound comprises an antisense oligonucleotide.

40. The composition of claim 38 wherein said first oligomeric compound comprises 10 to 40 nucleotides.

71. The oligomeric compound of claim 70 wherein each of said first and said second regions comprise at least 10 nucleotides.
72. The oligomeric compound of claim 70 wherein said first region in a 5' to 3' direction is complementary to said second region in a 3' to 5' direction.
73. The oligomeric compound of claim 70 wherein said oligomeric compound comprises a hairpin structure.
74. The oligomeric compound of claim 70 further comprising a third region located between said first region and said second region.
75. The oligomeric compound of claim 74 wherein said third region comprises at least two oligomeric residues.
76. The oligomeric compound of claim 74 wherein said oligomeric compound is RNA.
77. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to an internal residue of said oligomeric compound.
78. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to a terminal residue of said oligomeric compound.
79. The oligomeric compound of claim 78 wherein said terminal residue is at the 5' end of said oligomeric compound.
80. The oligomeric compound of claim 78 wherein said terminal residue is at the 3' end of said oligomeric compound.
81. The oligomeric compound of claim 70 wherein said conjugate moiety is attached to a heterocyclic base moiety of said oligomeric compound.
82. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to a monomeric subunit of said oligomeric compound.
83. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to an monomeric subunit linkage of said oligomeric compound.
84. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to said oligomeric compound through a linker.
85. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is a lipophilic moiety, vitamin, polymer, peptide, protein, nucleic acid, small molecule, oligosaccharide, carbohydrate cluster, intercalator, minor groove binder, cleaving agent, or cross-linking agent.
86. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is a steroid.
87. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is cholesterol or a cholesterol derivative.
88. The oligomeric compound of claim 70 wherein said at least one conjugate moiety binds to low-density lipoprotein.
89. The composition of claim 70 wherein said at least one conjugate moiety is folate and folate derivatives.
90. The composition of claim 70 wherein said at least one conjugate moiety is a water-soluble polymer.
91. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises polyethylene glycol or copolymer thereof.
92. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a fusogenic peptide or delivery peptide.
93. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a nuclear export signal.
94. The oligomeric compound of claim 70 wherein said at least one

7. The recombinant expression vector of claim 4 which is pSY3001 wherein the host is E. coli.

8. Host cells transformed with the expression vector of claim 3.

9. Host cells transformed with the expression vector of claim 4.

10. The recombinant DNA sequence of claim 1 wherein the conservative amino acid sequence is selected from the group consisting of alanine, serine, leucine, isoleucine, glutamate and valine.

11. The recombinant DNA sequence of claim 1, which DNA sequence encodes for an oxidation-resistant mutein selected from the group consisting of;

ala₁₀₄ ser₁₂₅ IL-2, ala₁₀₄ IL-2, ala₁₀₄ ala₁₂₅ IL-2, val₁₀₄ ser₁₂₅ IL-2, val₁₀₄ IL-2, val₁₀₄ ala₁₂₅ IL-2, des-ala₁ ala₁₀₄ ser₁₂₅ IL-2, des-ala₁ ala₁₀₄ IL-2, des-ala₁ ala₁₀₄ ala₁₂₅ IL-2, des-ala₁ val₁₀₄ ser₁₂₅ IL-2, des-ala₁ val₁₀₄ IL-2, des-ala₁ val₁₀₄ ala₁₂₅ IL-2, des-ala₁ des-pro₂ ala₁₀₄ ser₁₂₅ IL-2, des-ala₁ des-pro₂ ala₁₀₄ IL-2, des-ala₁ des-pro₂ ala₁₀₄ ala₁₂₅ IL-2, des-ala₁ des-pro₂ val₁₀₄ ser₁₂₅ IL-2, des-ala₁ des-pro₂ val₁₀₄ IL-2, des-ala₁ des-pro₂ val₁₀₄ ala₁₂₅ IL-2, des-ala₁ des-pro₂ des-thr₃ ala₁₀₄ ser₁₂₅ IL-2, des-ala₁ des-pro₂ des-thr₃ ala₁₀₄ IL-2, des-ala₁ des-pro₂ des-thr₃ ala₁₀₄ ala₁₂₅ IL-2, des-ala₁ des-pro₂ des-thr₃ val₁₀₄ ser₁₂₅ IL-2, des-ala₁

L7 47 S L4 AND (MALEIMID?)
L8 28 S L7 AND PY<2000

FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005

L9 59637 S (SERUM ALBUMIN)
L10 80 S L9 AND (CYS-34 OR CYSTEINE-34)
L11 13 S L10 AND AY<2000
L12 13 S L9 AND (ENHANCED PHARMACOKINETIC)
L13 11 S L12 NOT L10

=> file medline

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	84.52	112.35

FILE 'MEDLINE' ENTERED AT 20:27:40 ON 26 NOV 2005

FILE LAST UPDATED: 26 NOV 2005 (20051126/UP). FILE COVERS 1950 TO DATE.

On December 19, 2004, the 2005 MeSH terms were loaded.

The MEDLINE reload for 2005 is now available. For details enter HELP RLOAD at an arrow prompt (=>). See also:

<http://www.nlm.nih.gov/mesh/>
http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

OLDMEDLINE now back to 1950.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2005 vocabulary.

This file contains CAS Registry Numbers for easy and accurate substance identification.

=> s l1 and (enhanced pharmacokinetic)
258925 ENHANCED
40523 PHARMACOKINETIC
11 ENHANCED PHARMACOKINETIC
(ENHANCED(W) PHARMACOKINETIC)
L14 0 L1 AND (ENHANCED PHARMACOKINETIC)

=> s l1 and pharmacokinetic
40523 PHARMACOKINETIC
L15 434 L1 AND PHARMACOKINETIC

=> s l15 and (enhanced or increased or greater)
258925 ENHANCED
1214534 INCREASED
503507 GREATER
L16 144 L15 AND (ENHANCED OR INCREASED OR GREATER)

=> sl16 and py<2000
SL16 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter "HELP COMMANDS" at an arrow prompt (=>).

=> s l16 and py<2000
12397709 PY<2000
L17 103 L16 AND PY<2000

=> s l17 and albumin/ab
67629 ALBUMIN/AB
L18 86 L17 AND ALBUMIN/AB

=> s l18 (conjugat?)
MISSING OPERATOR 'L18 (CONJUGAT?)'
The search profile that was entered contains terms or nested terms that are not separated by a logical operator.

=> s l18 and conjugat?
78444 CONJUGAT?
L19 9 L18 AND CONJUGAT?

=> d l19,cbib,ab,1-9

L19 ANSWER 1 OF 9 MEDLINE on STN
2000163906. PubMed ID: 10701971. High sensitivity ELISA determination of

conjugate. The antiserum was coupled to cyanogen bromide-activated magnetisable cellulose, and antibody-bound and free fractions were separated by a simple magnetic device. A norethisterone/horseradish peroxidase **conjugate** was used as the label; o-phenylenediamine/hydrogen peroxide being the substrate for colour development. The results obtained by this direct EIA, which allowed processing of at least 100 samples per day, were compared with those of a well-validated enzymeimmunoassay featuring solvent extraction and centrifugal separation of antibody-bound and free steroid; the results were in excellent agreement (n = 30; r **greater** than 0.99) suggesting the usefulness of the simple high-throughput procedure for processing the large sample numbers generated by field investigations and **pharmacokinetic** studies.

=> d his

(FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005

L1 68146 S (SERUM ALBUMIN)
L2 74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3 39 S L2 AND PY<2000
L4 15041 S L1 AND PHARMACOLOG?
L5 1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6 2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7 47 S L4 AND (MALEIMID?)
L8 28 S L7 AND PY<2000

FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005

L9 59637 S (SERUM ALBUMIN)
L10 80 S L9 AND (CYS-34 OR CYSTEINE-34)
L11 13 S L10 AND AY<2000
L12 13 S L9 AND (ENHANCED PHARMACOKINETIC)
L13 11 S L12 NOT L10

FILE 'MEDLINE' ENTERED AT 20:27:40 ON 26 NOV 2005

L14 0 S L1 AND (ENHANCED PHARMACOKINETIC)
L15 434 S L1 AND PHARMACOKINETIC
L16 144 S L15 AND (ENHANCED OR INCREASED OR GREATER)
L17 103 S L16 AND PY<2000
L18 86 S L17 AND ALBUMIN/AB
L19 9 S L18 AND CONJUGAT?

=> log off

and a non-covalent mixture of rhG-CSF and HSA. RESULTS: The albumin-rhG-CSF conjugates are eliminated more slowly from the circulation. The clearance values are reduced from 0.839 +/- 0.121 ml/min/kg for rhG-CSF to 0.172 +/- 0.013 ml/min/kg for RSA-PEG-rhG-CSF and 0.141 +/- 0.005 ml/min/kg for HSA-PEG-rhG-CSF. WBC counts increased in both absolute number and duration as compared to rhG-CSF alone. The albumin rhG-CSF conjugates had enhanced serum stability relative to free rhG-CSF. The rate of degradation of the albumin conjugates incubated in rat serum at 37 degrees C decreased five fold. CONCLUSIONS: The results from the study show that specific conjugation of rhG-CSF to albumin decreases plasma clearance in vivo, causes increased WBC response, and increases serum stability as compared to free rhG-CSF.

Claim 38 (previously presented): The composition of claim 36 wherein said modified anti-viral peptide comprises the amino acid sequence of SEQ ID NO:2.

Claim 39 (previously presented): The composition of claim 36 wherein said modified anti-viral peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545.

Claim 40-55 (canceled)